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Fluorescent Properties of Daehwangjam, Golden Silk, and Juhwangjam and Their Diminishing upon HCl Vapor Exposure

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Abstract

For over five millennia, humans have benefited from the valuable byproducts of *Bombyx mori* silkworms nourished on mulberry leaves and a multitude of potential applications remains available due to the diverse array of silkworm varieties. In this work, we discuss the utilization of *Daehwangjam* (DHJ), golden silk (GS), and *Juhwangjam* (JHJ), distinctive colored silks found in Korea, as chemosensors. These novel silks emit fluorescence under external stimuli and show a diminishing fluorescence intensity when exposed to HCl vapor. The considerable surface-to-volume ratio of these cocoons allows for the identification of 5 ppm, 300 ppm, and 3000 ppm HCl vapors through decreased fluorescence intensity. The results show the suitability of natural DHJ, GS, and JHJ for applications in biosensing applications.

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Introduction

The cocoons of *Bombyx mori* silkworms have been used to manufacture high-quality smart textiles (Cherry, 1987). Textile platforms like these are sensitive to external stimuli, including pH, pressure, mechanical stress, temperature, light, and electrical signals (Stuart *et al*., 2010; Libanori *et al*., 2022; Chen *et al*., 2020). These textiles have porous structures with large surface-to-volume ratios that enhance the interaction between the textile and external stimuli as well as enhance the flexibility and permeability of the textile material (Hong *et al*., 2021; Guan *et al*., 2020). Integrating light manipulation into textile platforms allows for swift, wireless, and sensitive sensing operations (Zhang *et al*, 2023; Shin *et al*.; 2023). Specifically, fluorescent textiles can change color and exhibit illuminated patterns when exposed to light, providing protective features and suitability for applications like flexible displays, security barcodes, and sensory systems (Han *et al*., 2022; Lin *et al*., 2023). The use of toxic pigments and dyes in fluorescent textiles poses substantial risks to both humans and ecosystems (Tang *et al*., 2019; Jia *et al*., 2023). However, the emergence of natural textile materials offers a promising alternative, presenting a biocompatible and environmentally friendly option for smart textile applications (Vivekananthan *et al*., 2018; Tat *et al*., 2022).

Ongoing silkworm breeding aims to boost agricultural yields, yielding over 1000 varieties, including 340 pure breeds at Korea's National Institute of Agricultural Sciences (Goldsmith *et al*., 2004; Ji *et al*., 2017). However, the historical emphasis on fiber

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production has overshadowed the diverse silk varieties' untapped potential in biomedical applications (Gogurla *et al*., 2023; Min *et al*., 2017; Gogurla *et al*., 2019). The cocoon color depends on the silkworm variety (Lee *et al*., 2017) and these silkworm varieties exhibit color mutations, yielding golden, greenish, and pinkish cocoons influenced by carotenoids and flavonoids from mulberry leaves (Sakudoh *et al*., 2007; Hirayama *et al*., 2008; Kweon et al., 2012a; 2012b; Kim *et al*., 2020; Jeong *et al*., 2023). Carotenoids contribute to golden and pink hues, while flavonoids produce green colors, renowned for their potent antioxidant properties (Sakudoh *et al*., 2007; Hirayama *et al*., 2008). These pigments hold promise in bio-optics, demonstrating light absorption and emission at specific wavelengths.

This study explores the fluorescence properties observed in silkworm varieties (DHJ, GS, and JHJ) and assesses their potential for environmental sensing in textiles. The DHJ, GS, and JHJ silkworms naturally produce fluorescent pigments, notably carotenoids, in their sericin layer. Cocoon mats from these silkworm varieties were investigated as green-fluorescent materials under UV excitation (Kleinegris et al., 2010) and eventually as environmental sensors upon HCl vapor exposure. The results suggest a reduction in fluorescence intensity from the cocoon mats under HCl exposure based on the correlation of decay rate with exposure time and analyte concentration. Therefore, the present article outlines the natural cocoon fibers, rich in inherent pigments, as a sensitive platform for sensing applications.

Materials and Methods

Acquisition of silkworm cocoons

The three silkworm cocoon varieties used in this study were collected from the National Institute of Agriculture Sciences, Korea, including two silkworm varieties that produce yellow cocoons, GS (the F1 hybrid of pure breeding lines Jam 311 and Jam 312) and DHJ (the F1 hybrid of pure breeding line Jam 323 and Jam 324), and a silkworm variety that produce light pink cocoons, JHJ (the F1 hybrid of pure breeding lines 2303 and BP Heehong).

Preparation of silkworm cocoon mats

Pristine cocoon mats were prepared by drying and cleaning the cocoons with an N_2 gun and then cutting them into 1 cm × 1 cm pieces and flattening them. Degummed fibers mats were prepared by boiling the dried cocoons for 30 min in an aqueous solution of 0.02 M Na_2CO_3 removing globular sericin proteins, rinsing the extracted silk fibroin fibers three times with deionized water for 20 min, storing the prepared silk fibroin fibers in 1 cm × 1 cm molds, drying the degummed fibers for 24 h, and flattening them.

Preparation of HCl vapor environment

To generate an HCl vapor environment at a parts per million (ppm) scale, liquid HCl was dispensed into a beaker using a micropipette. Subsequently, the beaker was sealed for 2 h at room temperature to allow complete vaporization of the liquid HCl within. To achieve a concentration of 1 ppm (v/v), the ratio of the volume of HCl vapor to the volume of the beaker was maintained at 1 μL/L. HCl vapor atmospheres were created at 5 ppm, 300 ppm, and 3000 ppm, respectively.

Optical measurements and characterizations

The fluorescence spectra of pristine and degummed fibers from DHJ, GS, and JHJ were recorded using a visible/near-infrared spectrometer (USB-2000+ VIS-NIR-ES, Ocean Optics, USA, spectral resolution: 1.2 nm). A 365-nm continuous-wave LED (M365LP1 UV Mounted LED, Thorlabs Inc., USA) was employed to optically illuminate the samples. To cover a larger area on the samples, a plano-convex optical lens (f = 10 cm) was used to expand the illuminating spot of the LED. High-quality DHJ, GS, and JHJ pristine and degummed fibers were created, ensuring spatial uniformity in diameter and fluorescence emissions. The spectrometer detector tip's precise distance from the sample was adjusted, maintaining the focused beam size, and the detection angle was fixed by positioning the detector tip perpendicular to the sample.

Results and Discussion

The National Institute of Agricultural Sciences in Korea provided three silk cocoon varieties: DHJ, GS, and JHJ. Figs. 1a-c show images of DHJ (yellow), GS (yellow), and JHJ (light pink) cocoons, under natural light, respectively, where the silkworm-produced pigments, carotenoids, control the yellowish and pinkish colors of these cocoons. Mulberry leaf nutrients cause all silk varieties to exhibit fluorescence under UV light (Fig.

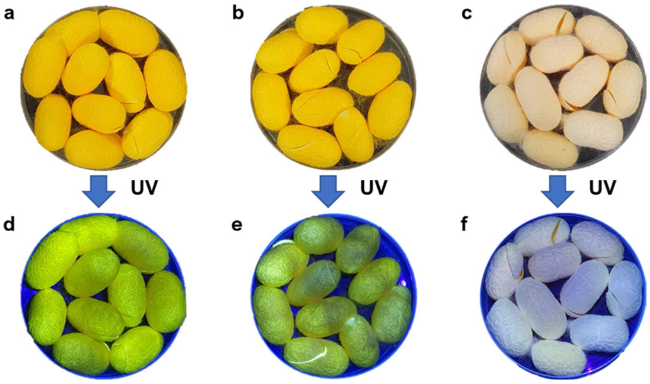

Fig. 1. Photograph images of (a and d) *Daehwangjam* (DHJ), (b and e) golden silk (GS), and (c and f) *Juhwangjam* (JHJ) cocoons under daylight and UV light, respectively.

1d-f). While the JHJ cocoons display a faint green fluorescence, GS cocoons show green fluorescence and DHJ cocoons show increased green fluorescence.

To quantitatively examine fluorescence, a 365-nm light-emitting diode (LED) emitted UV light, mounted on the spectrometer setup, was collimated and directed onto silk cocoon samples. The resulting fluorescent light was collected by an optical fiber and transmitted to the spectrometer for recording fluorescent spectra. Figs. 2a-c display the normalized fluorescent spectra of DHJ, GS, and JHJ cocoon mats. Notably, a gradient from strong to faint green fluorescence was observed in the order of DHJ, GS, and JHJ cocoon mats. Given that silk fiber comprises two proteins (fibroin in the core and sericin in the sheath), determining the location of the fluorescent pigment is crucial. PS fiber mats were completely degummed to eliminate the outer sericin layer and were then excited by the UV LED. As depicted in Figs. 2a-c, the fluorescence vanished post-degumming, indicating that the pigment molecules were predominantly located inside the sericin layer (Ma *et al*., 2016).

The large surface-to-volume ratios of DHJ, GS, and JHJ cocoons make them ideal for extensive application as fluorescent gas sensors. HCl vapor was selected as the analyte due to its widespread use in organic compound production, despite being corrosive and harmful to humans. Lethal concentrations (LCLo) of HCl vapors within 30-minute and 5-minute exposures are 1300 and 3000 ppm, respectively (Lestari *et al*., 2005). The RD_{50} test, indicating a 50% decrease in respiratory rate, reports a 10-minute value for mice at 309 ppm (Barrow *et al*., 1977). Furthermore, the permissible exposure limit for HCl vapor in workplaces over an 8-hour time-weighted average (TWA-PEL) is set at 5 ppm (Baron *et al*., 1996), significantly lower than lethal concentrations for prolonged exposure.

To evaluate the gas-sensing response of fluorescent DHJ, GS, and JHJ, these cocoons were exposed to controlled concentrations of HCl vapors in a beaker. Fig. 3a-i depicts the emission spectra of the cocoons during up to a 5-minute exposure to HCl vapors. For quantitative analysis, the cocoon mats were exposed to various HCl vapor concentrations (ranging from 5 to 3000 ppm). Exposure to a strong acid (300 ppm) caused a rapid decline in fluorescence intensities, indicating potential for fluorescent chemosensing. HCl vapor exposure resulted in a gradual fading of daylight color and fluorescence. After a 5-minute exposure, the fluorescence intensity for the 5 ppm HCl exposed cocoon mats reached 71.33% for DHJ, 85.75% for GS, and 59.88% for JHJ. Similarly, after a 5-minute exposure, the fluorescence intensity for the 300 ppm HCl exposed cocoon mats reached 18.77% for DHJ, 31.57% for GS, and 30.75% for JHJ. Finally, for the same 5-minute exposure, the fluorescence intensity for the 3000 ppm HCl exposed cocoon mats reached 9.80% for DHJ, 30.92% for GS, and 18.20% for

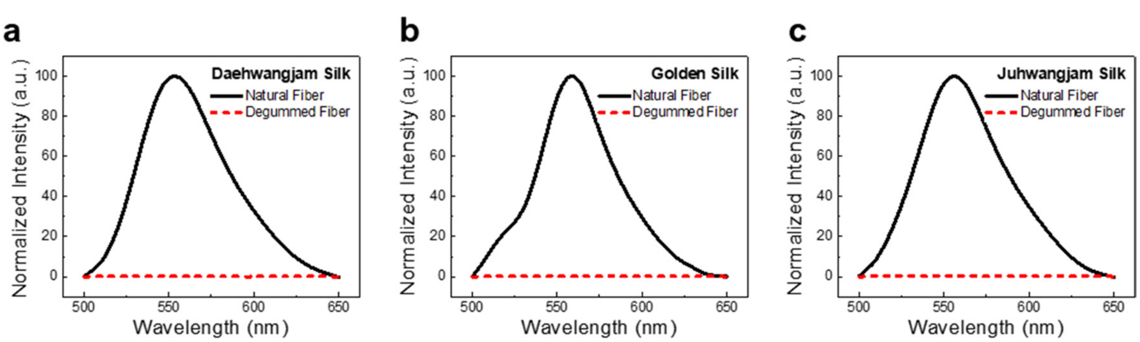

Fig. 2. Emission spectra of natural and degummed fibers from (a) DHJ, (b) golden silk (GS), and (c) JHJ, respectively.

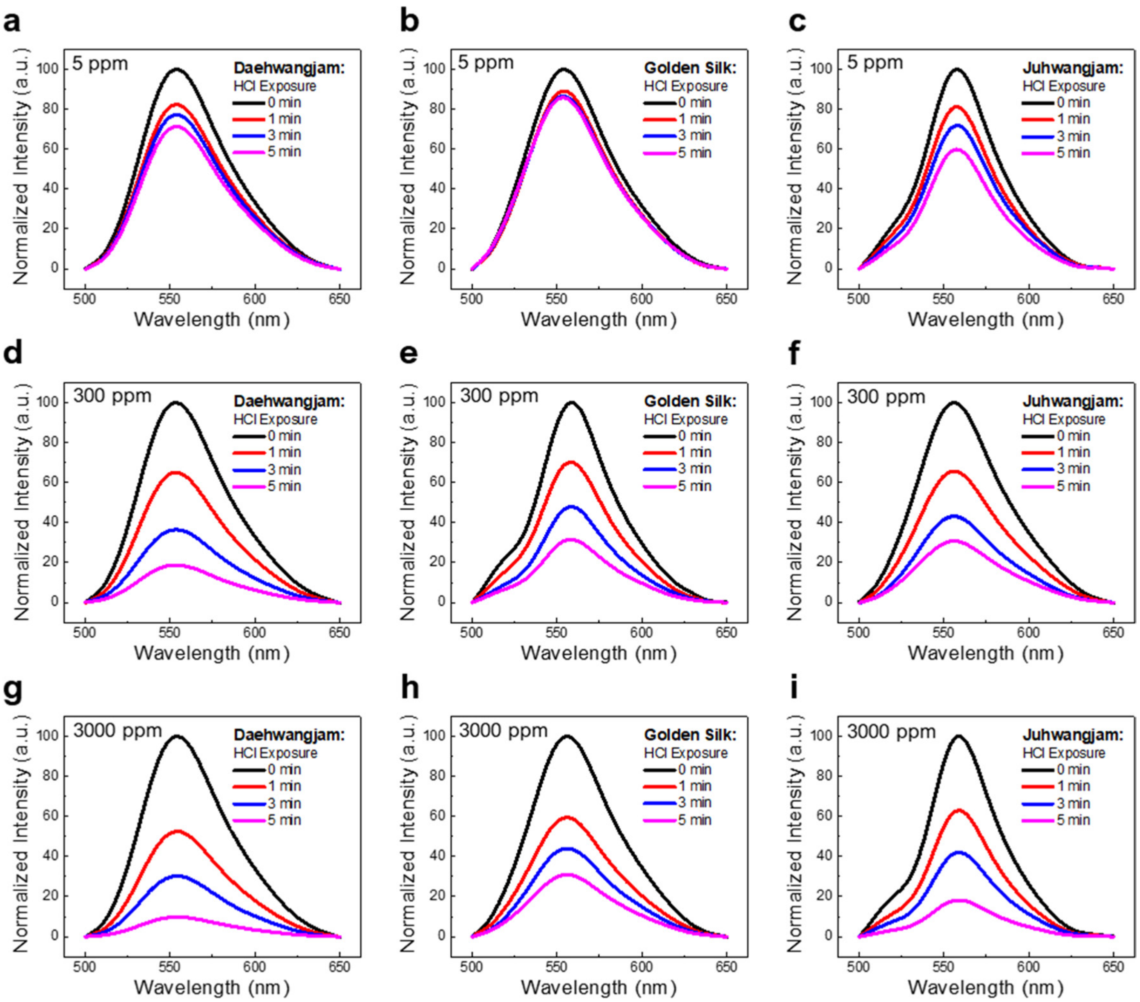

Fig. 3. Time-dependent emission spectra from (a, d, g) DHJ, (b, e, h) golden silk (GS), and (c, f, i) (JHJ) cocoon mats under 5 ppm, 300 ppm, and 3000 ppm HCl vapor exposures, respectively.

JHJ. Remarkably, fluorescence decayed linearly and would eventually disappear at all HCl vapor concentrations, with the fading rate depending on the HCl concentrations.

Conclusion

In summary, our study demonstrates the efficacy of utilizing yellow DHJ and GS cocoons, as well as light pink JHJ cocoons, as fluorescent environmental sensors. These cocoon mats exhibit varying intensities of green fluorescence, progressing from light green to a strong green color in the sequence of JHJ to GS to DHJ. The identified fluorescent pigment molecules are carotenoids located in the outer sericin layer. DHJ, GS, and JHJ cocoon mats exhibit prompt responsiveness and high sensitivity to HCl vapors. Even at low concentrations (a few ppm) of HCl vapors, noticeable changes in fluorescence intensity occur, and at a lethal concentration (3000 ppm), an immediate decrease in fluorescence intensity is observed. Additional investigation is required to completely understand the light-emitting characteristics of carotenoid-based pigments in DHJ, GS, and JHJ cocoons. Nevertheless, incorporating these natural fluorescent pigments into fabrics and health-promoting dietary supplements holds potential as materials suitable for optical sensing applications that are both environmentally friendly and biocompatible.

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