Establishment of Dyeing Data for Silk Fabrics and Cells Using *Diospyros kaki* Thunb

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감나무 열매를 이용한 실크 및 세포에 대한 염색 데이터 확립

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Abstract In this study, it was analyzed with the dyeing pattern of *Diospyros kaki* Thunb (persimmon) and was tried to numerically evaluate how the dyeing pattern in silk fabrics and cells was changed by different mordants. When the dyed silk fabrics were sufficiently dried, the silk fabrics were found to have a pale yellow color. Interestingly, iron II sulfate mordant changed the color change the most, silk fabrics were dyed with a color close to brown or dark purple. For numerical analysis, 19% and 62.5% color changes could be induced by sodium tartrate plus citric acid and copper acetate, respectively. Iron II sulfate induced the greatest difference than that of untreated mordants at 88%. About 5% and 10% of Chinese hamster ovary (CHO) cells were stained by sodium tartrate plus citric acid and copper acetate, respectively. The staining effect induced by iron II sulfate was about 2.4 times higher than the staining effect by sodium tartrate plus citric acid. In previous studies, staining results have been visually confirmed. However, this results not only visually confirmed the dyeing, but also quantified the color change. In particular, if numerical results are continuously integrated into big data, any researcher will be able to easily obtain similar results even if the method, time, volume, etc. are changed. In addition, the numerical data of this study is considered to be an important basis for building a database for IoT construction and computer analysis.

Key Words : Dyeing, persimmon, IoT, Color change, Mordant

요 약 본 연구에서는 *Diospyros kaki* Thunb(감)의 염색 패턴으로 분석하여 매염제에 따라 견직물과 세포의 염색 패턴이 어떻게 변화하는지 수치적으로 평가하고자 하였다. 염색된 견직물이 충분히 건조되었을 때 견직물은 엷은 황색을 띠는 것으로 나타났다. 흥미롭게도 철 II 황산염 매염제는 색 변화를 가장 많이 변화시켰고, 견직물은 갈색 또는 암자색에 가까운 색으로 염색되었다. 수치해석을 위해 타르타르산 나트륨과 구연산 및 아세트산 구리에의해 각각 19%와 62.5%의 색상 변화가 유도될 수 있었다. 철 II 황산염은 무처리 매염제보다 88%로 가장 큰 차이를 보였다. 중국 햄스터 난소(CHO) 세포의 약 5% 및 10%는 각각 타르타르산나트륨과 시트르산 및 아세트산구리로 염색되었다. 철 II 황산염에 의해 유도된 염색 효과는 타르타르산 나트륨 + 시트르산에 의한 염색 효과보다 약 2.4배 더 높았다. 기존의 연구들에서는 염색 결과를 육안적으로 확인하여왔다. 하지만, 본 논문의 결과들은 염색을 육안적으로 확인할 뿐만아니라 색상변화를 수치화한 것이다. 특히, 빅데이터에 수치화된 결과들이 계속 집약 된다면 방법에서, 시간, 부피 등을 변화시키더라도 유사한 결과들을 어느 연구자들이 쉽게 얻을 수 있을 것이다. 또한, 본 연구의 수치 데이터는 IoT 구축 및 컴퓨터 분석을 위한 데이터베이스 구축에 중요한 근거가 될 수 있을 것으로 사료된다.

주제어 : 염색, 감, 사물인터넷, 색변화, 매염제

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1. Introduction

Natural dyeing has been widely used in daily life for a long time, and as various natural dyes are developed, dyeing patterns are also diversifying [1]. However, since it is difficult to accurately evaluate the reproducibility of dyeing results due to the exact amount and treatment time of dyeing materials and mordants, it is necessary to establish more stringent dyeing data. Natural dyes refer to ingredients derived primarily from plants, insects, etc. and can be used directly, indirectly or in combination with mordants [3]. The biggest advantage of natural dyes is that it can reduce health-related problems, such as allergies, compared to synthetic dyes, so it can be used very usefully to a variety of living and scientific areas [3].

Diospyros kaki Thunb is mainly cultivated in Korea, China and Japan, especially in the southern regions of Korea [4]. Persimmons are sweet and has a hard skin, and when dried they loses moisture and are even sweeter [5]. Astringent persimmons regulate cholesterol accumulation biologically by inhibiting oxidative stress and regulating the levels of LDL and HDL-related genes [6].

Biological evaluation using persimmon extract has been performed in vivo rats [7]. In laboratory animals whose diarrhea was induced by oxidative stress. Antioxidant and biochemical effects of *D. kaki* fruit aqueous extract administered significantly reduced gastrointestinal transit and intestinal fluid accumulation, depending on the administered dose. In addition, as a result of pre-treatment of the extract, it was confirmed that oxidative stress status deregulation induced by carbon monoxide (CO) intoxication was also reduced.

In another study, it was confirmed that the acetone extract of *D. kaki* leaves has an effect of promoting the differentiation of cancer cells, and therefore, a study suggesting that it could be applied to the treatment of acute promyelocytic leukemia has also been reported [8]. Persimmon peel extract is a non-toxic and biodegradable alternative to sodium dithionite and can be used for indigo reduction dyeing [9]. However, there are still few reports of natural dyeing for persimmon. In addition to persimmon, it is difficult for other researchers to obtain accurate dyeing results due to the lack of data that converts the exact results of the staining degree into numbers for natural dyes. In addition, due to the lack of various data, cases where natural dyeing and natural dyeing results are applied to the Internet of Things (IoT) are still rare worldwide [10]. In the IoT environment, since everything connected by a network, such as people, objects, and spaces [11], can be connected in real time, accurate information on dyeing results can be applied to IoT.

Various colors can be induced by adding a mordant to natural dyes [12]. In addition to mordants, color can also be induced by the concentration of hydrogen ions in the solution. Therefore, various conditions, such as the concentration of the mordant and the treatment time, induce the color, and it is necessary to quantify the color to establish accurate data. In this way, if all dyeing data is digitized and applied to the IoT, the dyeing changes caused by various natural dyes can be accurately evaluated. In addition, the digitization of dyeing patterns due to the combination of dye and mordant can also be a basic data that can be applied to IoT.

It is very easy to simply visually check the change in staining. However, it is not easy for anyone to obtain the same result using this research method. Therefore, it is necessary to quantify the results of this study. Therefore, in this study, it was analyzed with the dyeing pattern of persimmon and was tried to

numerically evaluate how the dyeing pattern was changed by different mordants. In addition, by applying dyed silk to cells and analyzing how the staining changes, it was tried to evaluate whether changes in fabrics and cells could be applied in terms of biotechnology.

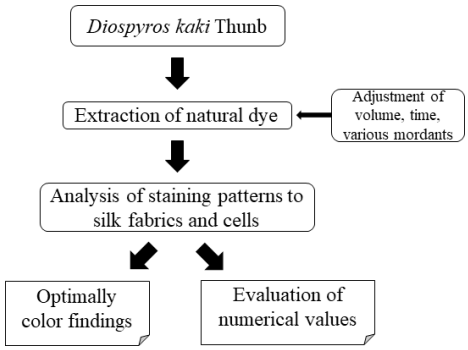
2. Materials and Methods

2.1 Extraction of natural dye from *D. kaki* Thunb and cultivation of Chinese hamster ovary (CHO) cells

The dye extraction method was carried out by slightly modifying a previous report [10]. The staining pattern is very likely to change if existing methods are altered. In addition, dyeing patterns can be very diverse by treatment with a mordant. Therefore, in this research method, the existing method was used, but the time, volume, and pH were slightly changed. One hundred gram of blue persimmon was put in a large mortar and pounded well for 30 min to extract enough extract from persimmon. Then, 900 ml of water was added and the persimmon was pounded again for 10 min. The above method was carried out at room temperature and the pH of the aqueous solution was maintained at 7. Using a sieve, the extract from the above was filtered twice so that there were almost no foreign substances. Any undissolved foreign matter that was visually confirmed and present was filtered again. The dye extracted in this way was stored at 4℃ to prevent contamination, and was taken out at room temperature for later dyeing and maintained for 30 min. Briefly, CHO cells were cultured in a 24-well culture plate at 5×10^4/well and maintained at 37°C with 5% carbon dioxide [13]. For culture, Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL) was used and staining was performed in a 24-well culture plate.

2.2 Application of mordants and dye to silk fabric and cells

In this study, the mordants, e.g., iron II sulfate, sodium tartrate plus citric acid, copper acetate were applied to silk fabric [14]. It was dyed by adding silk fabrics to 900 ml of the extracted dye. In the first attempt, silk fabrics were dyed for 20 minutes. The silk was left in the dye for an additional 20 min so that the silk fabrics, which had not been treated with any mordants, could be visually dyed with very distinct colors. The dyed silk fabrics were sufficiently dried in the sun for 10 days to completely remove moisture. For mordant treatment, iron II sulfate (20 gram), sodium tartrate plus citric acid (30 gram plus 90 gram), and copper acetate (10 gram) were added to 900 ml of dye for 30 min, so that the mordant was sufficiently combined with the dye. Silk fabrics treated with mordants and dyes were dried in the same manner as above. On the other hand, in order to dye the silk fabrics to CHO cells, the silk fabrics were placed in a glass bottle and autoclaved. Thereafter, the dyed silk fabrics were cut with scissors to fit one well of the 24-well culture plate in which CHO cells were cultured, put into the well, and dyeing proceeded for 1 hr. The overall experimental flow chart is illustrated in Figure 1.



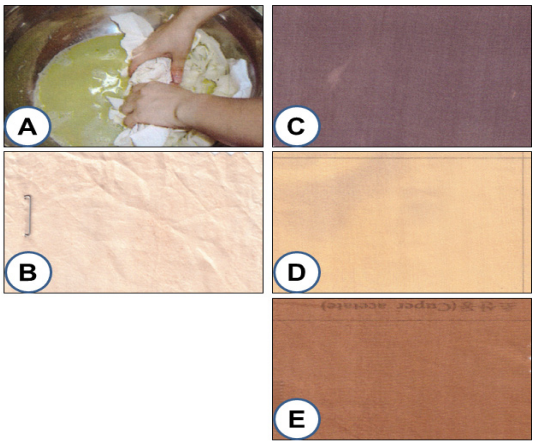
[Fig. 1] Flow chart of overall experimental methods.

3. Results

3.1 Natural dyeing of silk fabrics by *D. kaki* Thunb

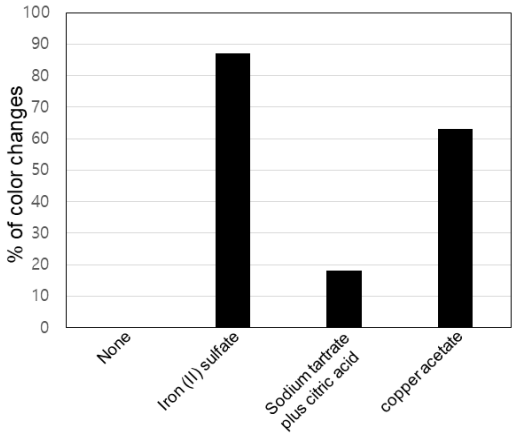
In order to analyze the natural dyeing pattern by dye from *D. kaki* Thunb, its dye was added to silk fabrics (Fig. 2). In addition, it was also observed how the staining pattern by various mordants changes. It is considered important to visually analyze the color change of dyeing, but it is also considered very important to have a large amount of accurate information by quantifying the dyeing pattern.

When the dyed silk fabrics were sufficiently dried, the silk fabrics were found to have a pale yellow color (Fig. 2B). When the mordant of sodium tartrate plus citric acid was added, silk fabrics were dyed in a slightly darker yellow color, so the color change caused by sodium tartrate plus citric acid did not show much (Fig. 2D). However, iron II sulfate mordant changed the color change the most, silk fabrics were dyed with a color close to brown or dark purple (Fig. 2C). On the other hand, copper acetate dyed silk fabrics between yellow and brown or brown (Fig. 2E).



[Fig. 2] Staining patterns by dyes from *D. kaki* Thunb and mordants to silk fabrics. Three mordants were applied. A showed rubbing step of silk fabrics by addition of dyes. B, C, D and E represented the staining patterns by adding no mordant, iron II sulfate, sodium tartrate plus citric acid and copper acetate, respectively.

As mentioned above, it is considered very necessary to quantify the staining pattern to construct specific information. When silk dyeing by only *D. kaki* Thunb-dye was set to 0% without mordant treatment, about 19% and 62.5% color changes could be induced by sodium tartrate plus citric acid and copper acetate, respectively (Fig. 3). Iron II sulfate, which induced the greatest color change, induced the greatest difference than that of untreated mordants at 88% (Fig. 3).

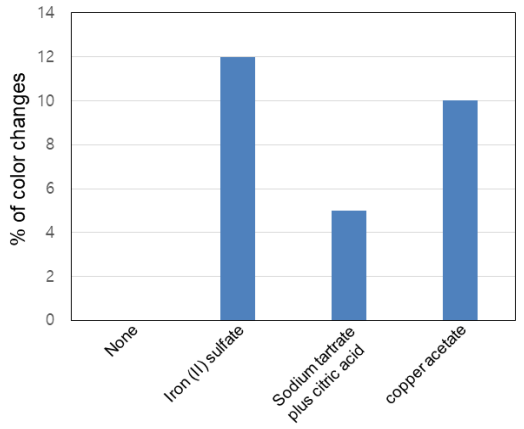


[Fig. 3] Quantification of staining patterns by dyes from *D. kaki* Thunb and mordants to silk fabrics. None indicated staining patterns without any mordant.

3.2 Application of natural dyes from *D. kaki* Thunb to CHO cells

After dyeing with *D. kaki* Thunb-dye and a mordant, the dried silk fabrics were analyzed for any effect on cell staining (Fig. 4). CHO cells have been particularly frequently used for cytotoxicity and the like [13]. Natural dyes must be non-toxic, so CHO cells were used in this study as in the previous method. After cutting the silk to the size of the well of the 24-well culture plate, it was added to the well and the staining pattern was digitized. When compared with the figure 2, the difference was very cut, but

the staining effect on cells was not great. When the group not treated with the mordant was 0%, about 5% and 10% of CHO cells were stained by sodium tartrate plus citric acid and copper acetate, respectively. Staining was most intense by iron II sulfate, showing a difference of 12% compared to the negative control. The staining effect induced by iron II sulfate was about 2.4 times higher than the staining effect by sodium tartrate plus citric acid.



[Fig. 4] Quantification of staining patterns by dyes from *D. kaki* Thunb and mordants to silk fabrics. None indicated staining patterns without any mordant.

4. Discussion

Extracted natural dyes can be more stable to health than using chemical dyes in humans or animals [15,16]. There are many different ways to extract natural dyes. In general, natural dyes are extracted by adding a large amount of water to crush and press natural materials. In order for the extracted dye to be relatively harmless to the human body, water is generally added.

In this study, a sufficient amount of water was used to extract dyes from *D. kaki* Thunb and various mordants were used to induce changes in silk fabrics staining. In addition, the extracted dye solution was maintained at pH 7. When the dyed silk fabrics were placed on top of the CHO cells, it was confirmed that the dye transferred to the cells, but the staining was not very distinct. However, as in silk fabric, iron II sulfate was observed to have the greatest effect on dye transfer into CHO cells.

Aluminum, iron, copper, tin, etc. are mainly used as metal components of the mordant [17]. The iron II sulfate mordant used in this study has iron as a metal component and induces the greatest effect on silk fabrics and cell dyeing. However, the dyeing effect by the sodium tartrate plus citric acid mordant containing citric acid, an acid component, was not very large. Taken together, among various mordants, it is thought that the metal component has the greatest effect on dyeing change. Similar to the results of this study, in a previous study, dyeing of silk fabrics by dyes extracted from *Lithospermum* erythrorhizon induced a difference of about 75% by iron II sulfate [10]. In this study, the dye was filtered using a sieve. However, it is believed that using a 0.45 μm-sized syringe filter would increase the purity of the dye and help to quantify a more accurate dyeing pattern [1]. In addition, the digitization of dyeing patterns was studied by mixing mordants with such natural dyes. In particular, the highest color change was induced when 15 grams of copper acetate was mixed with the dye extracted from *Phellodendron amurense Ruprecht* [1]. Interestingly, in this study, the color change was very distinct by 10 grams of copper acetate. The pH of the staining solution used in this study was 7. However, it was assumed that the hydrogen ion concentration of the dyeing solution was changed from pH 7 to slightly acidic by the mordant, especially citric acid, which induced an acidic state.

This study aimed to analyze the dyeing

patterns on silk fabrics and cells by natural dyes. In particular, setting an accurate protocol and result values that can be applied to IoT networks by expressing dyeing changes numerically will create an environment where anyone can dye more accurately [18]. According to the most recent research report, there is real-time dyeing of fabrics using dyeing methods and IoT-based application cases [19]. In previous studies, staining results have been visually confirmed. However, this results not only visually confirmed the dyeing, but also quantified the color change. In particular, if numerical results are continuously integrated into big data, any researcher will be able to easily obtain similar results even if the method, time, volume, etc. are changed.

Overall, this study aimed to build more accurate data by quantifying the dyeing changes by applying natural dyes to silk fabrics and cells using various mordants. There will still be many barriers to the application of IoT for natural dyes. However, it is thought that the results of this study can serve as important evidence for building a database for IoT construction and computer analysis.

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