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Rapid analysis of dyed safflowers by color objectification and pattern recognition methods

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Abstract *Objective:* Rapid discrimination of three classes of safflowers, dyed safflower, adulterated safflower, and pure safflower using computer vision and image processing algorithms.
Methods: A low cost computer vision system (CVS) was designed to measure the color of safflowers in the RGB (red, green, blue), $L^*a^*b^*$, and HSV (hue, saturation, vale) color spaces. The color moments in these three color spaces were extracted from the acquired images as color features of safflower. In addition, five kinds of pigments that are commonly used to dye safflowers were identified by high-performance liquid chromatography as a reference. Pattern recognition methods were investigated for rapid discrimination, including an unsupervised principal component analysis (PCA) algorithm and a supervised partial least squares discriminant analysis (PLS-DA) algorithm.
Results: The mean error ($\bar{e}$) between color values measured with the colorimeter and calculated with the CVS was 2.4%, with a high correlation coefficient (r) of 0.9905. This result indicated that the established CVS was reliable for color estimation of safflowers. The PLS-DA model, which had a total accuracy of 91.89%, outperformed the PCA model in classifying pure, adulterated, and dyed safflowers.

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Conclusion: The color objectification is a promising tool for rapid identification of dyed and adulterated safflowers.

Introduction

Safflower (*Carthamus tinctorius* L.) is a well-known Chinese herbal medicine (CHM). It has been used clinically for the treatment of cardiovascular, cerebrovascular, and gynecological diseases for centuries.[1] Safflower is cultivated worldwide mainly for its seeds, which contain edible oil with recognized health benefits.[2] Safflower petals are also used as a food additive and a natural pigment.[3] The main active ingredient of safflower is Carthamus yellow, which is composed of safflomin A and safflomin B.[4]

However, the safety of safflower use has been challenged because it is often the target of artificial coloration and adulteration. The color of safflower is usually associated with the content of the active pharmaceutical ingredient, which makes it an important attribute for determining the quality of safflowers. Safflowers with poor quality and light color are often dyed using artificial synthetic pigments and industrial dyes. Most of these dyes are potential carcinogens that can greatly damage human health. Therefore, appropriate approaches are needed to identify artificially colored safflowers.

Several analytical methods have been used to detect pigments, such as high-performance liquid chromatography (HPLC) and mass spectrometer (MS). Mazzetti et al developed a HPLC method for the identification and determination of Sudan I in chili powder.[5] Ma et al used a HPLC-MS method for the simultaneous determination of water-soluble and fat-soluble synthetic dyes, including Tartrazine, Amaranth, Ponceau 4R, Sunset Yellow FCF, Brilliant blue FCF, and Sudan (I—IV), in foodstuffs.[6] Xiao et al identified six adulteration dyes in *C. tinctorius* (also known as Carthami Flos), including Tartrazine, New Coccine, Sunset Yellow FCF, Azorubin, Acid Red 73, and Orange II, using thin-layer chromatography, HPLC, and LC-MS.[7] However, these methods all involve sample preparation processes, which can be time-consuming and tedious.

Expert knowledge is a commonly used approach to identify the authenticity of CHM. The inner quality of CHM is defined by several physicochemical characteristics, such as color, size, shape, smell, and texture. However, color information can be fuzzy and inconsistent because decisions made by expert operators are subjective and can be affected by psychological factors such as fatigue or acquired habits. In addition, consumers who lack expertise cannot easily identify dyed products, which often have only small color differences. Thus, misclassification between authentic and dyed products is common.

This problem can be prevented if automated inspection systems based on computer vision that incorporate pattern recognition methods are used. Computer vision systems (CVSs) can provide large amounts of information about the attributes of objects, which can improve the capabilities of human inspection.[8] The advances in computer and image processing techniques mean that CVSs are now available for quality inspection of many agricultural products. Kang et al applied computer vision to provide quantitative descriptions of the color profiles and color changes of mango using hue angle.[9] Color features have been used to distinguish between defective and good tomatoes using computer vision combined with a rule-based approach and a neural network method.[10] The overall accuracy of these two methods was 84% and 87.5% respectively. However, no studies that focus on the coloration and adulteration of CHM have been reported so far.

The aim of the present study is to preliminarily investigate the performance of color objectification for the identification of safflower coloration and adulteration. A CVS was designed to measure the color of safflower based on RGB, $L^*a^*b^*$, and HSV color spaces. The color moments were extracted from the acquired digital images as color features. Pattern recognition methods, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were tested for discrimination of safflowers. Besides, the CVS integrated image acquisition, image processing, and decision making using an in-house program written in MATLAB language.

Materials and methods

Materials

Neucoccin, Sunset Yellow, Acid Red 73, Orange II, and Auramine O standards for identification were purchased from the National Institutes for Food and Drug Control (Beijing, China). Analytical grade ammonium acetate and ethanol were obtained from the Beijing Chemical Works (Beijing, China). Chromatographic grade acetonitrile and methanol were purchased from Thermo Fisher Scientific (Waltham, MA). Deionized water was purified using a Milli-Q water system (Millipore Corporation, Boston, MA).

Seven batches of safflower were collected from different areas in China. One batch was identified as dyed safflowers with pigments; and the other six batches were pure safflowers. Besides these seven batches, four adulterated sample sets were prepared by mixing the dyed safflowers with the pure ones in different proportions (5%, 10%, 15%, and 20%). Ten samples were drawn randomly from each batch for analysis and a total of 110 samples were obtained.

HPLC qualitative analysis of dyed safflower

The authenticity of each safflower sample was determined by HPLC using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, Cuba). The system was equipped with a quaternary pump, a vacuum degasser, an autosampler, and