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Honey characterization using computer vision system and artificial neural networks

Sahameh Shafiee [a], Saeid Minaei [a,*], Nasrollah Moghaddam-Charkari [b], Mohsen Barzegar [c]

[a] Department of Agricultural Machinery Engineering, Tarbiat Modares University, Tehran, Iran
[b] Department of Electrical and Computer Engineering, Tarbiat Modares University, Tehran, Iran
[c] Department of Food Science and Technology , Tarbiat Modares University, Tehran, Iran

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ABSTRACT

This paper reports the development of a computer vision system (CVS) for non-destructive characterization of honey based on colour and its correlated chemical attributes including ash content (AC), antioxidant activity (AA), and total phenolic content (TPC). Artificial neural network (ANN) models were applied to transform RGB values of images to CIE L*a*b* colourimetric measurements and to predict AC, TPC and AA from colour features of images. The developed ANN models were able to convert RGB values to CIE L*a*b* colourimetric parameters with low generalization error of 1.01 ± 0.99. In addition, the developed models for prediction of AC, TPC and AA showed high performance based on colour parameters of honey images, as the R^2 values for prediction were 0.99, 0.98, and 0.87, for AC, AA and TPC, respectively. The experimental results show the effectiveness and possibility of applying CVS for non-destructive honey characterization by the industry.

1. Introduction

Since the earliest times, honey has been used as food, food additive, food preservative and medicine. It is the natural sweet substance produced from the collected nectar of blossoms and exudates of trees or plants by *Apis mellifra* bees (Alvarez-Suarez, Tulipani, Romandini, Vidal, & Battino, 2009). Honey contains at least 181 substances (Chow, 2002). It is composed of fructose (38%), glucose (31%), minerals, proteins, free amino acids, enzymes and vitamins (Pérez, 2002; Terrab et al., 2003). Also, a wide range of minor ingredients are present in honey, many of which are known to have antioxidant properties, such as phenolic acids (Dimitrova, Gevrenova, & Anklam, 2007; Martos et al., 2000; Tomas-Barberán, Martos, Ferreres, Radovic, & Anklam, 2001). Honey is rich in natural antioxidants and minerals. Thus, it takes a more active role to make a contribution to human health and nutrition. Honey has a wide range of colours from water white to dark amber or dark. Honey colour depends on various factors, mineral content being an important one. Light-coloured honeys usually have low ash content. On the contrary, dark-coloured honeys generally have higher ash contents (Al et al., 2009; Gomes, Dias, Moreira, Rodrigues, & Estevinho, 2010). The evidence of the biological actions of honey can be ascribed to its polyphenolic contents which, in turn, are usually associated with its antioxidant and anti-inflammatory actions, as well as its cardiovascular, antiproliferative and antimicrobial benefits (Alvarez-suarez, Giampieri, & Battino, 2013). Some research groups have shown that the total phenol and flavonoids contents, and antioxidant activity of honey are greatly dependent on colour (Alvarez-Suarez et al., 2010; Bertoncelj, Doberšek, Jamnik, & Golob, 2007; Estevinho, Pereira, Moreira, Dias, & Pereira, 2008; Ferreira, Aires, Barreira, & Estevinho, 2009). Therefore, colour is an important feature in quality control of honey. The quality parameters (i.e., antioxidants) of honey are normally measured using conventional analytical techniques (Alvarez-Suarez et al., 2009). However, applying these techniques in honey industry has some disadvantages such as their destructive nature, implementation expense, and a time requirement. For food authentication and honey characterization in particular, introduction of alternative methods to conventional procedures aiming at objective measurement of honey in a consistent and cost effective manner is of great importance for the honey industry (Shafiee, Minaei, Moghaddam-Charkari, Ghasemi-Varnamkhasti, & Barzegar, 2013). In this regard, several studies have been carried out to develop nondestructive techniques using a calibration data set obtained by analytical methods for various agricultural products (Brosnan & Sun, 2004; Butz, Hofmann, & Tauscher, 2005; Pace et al., 2013; Ruiz-Altisent et al., 2010). Among these approaches, computer vision can be noted for having good capability of food colour assessment as well as food characterization for those properties associated with

* Corresponding author. Tel.: +98 2148292466; fax: +98 2148292200.
E-mail address: minaee@modares.ac.ir (S. Minaei).

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colour. Many research groups have used computer vision for analysis of food colour. The first step in the development of a computer vision technique for food quality assessment based on colour is colour calibration and characterization. Briones and Aguilera (2005) employed image analysis techniques to analyse colour variations during blooming development in chocolate. They concluded that computer vision systems have the capability to quantify overall changes as well as particular features over the whole chocolate surface. Thus, they introduced their vision based approach for customization and standardization of quality assessment of chocolate. Zheng et al. (2011) proposed a method to predict the changes of anthocyanins, ascorbic acid, total phenols, flavonoids, and antioxidant activity of red bayberry juice using CVS and ANN combination. In a recent study, CVS has been utilized to predict antioxidant activity and total phenols in pigmented carrots (Pace et al., 2013). It was demonstrated that CVS can act as an effective colour assessment tool and act as a proper predictor for antioxidant properties of carrot pigments. On the other hand, there is no report that employs CVS for predicting honey antioxidant activity, total phenolic content, and ash content.

The objective of this research was to study the relationships between TPC, AA, AC, and honey colour. Moreover, we aim to evaluate the effectiveness of CVS–ANN combination for honey colour assessment and prediction of its TPC, AA, and AC.

2. Materials and methods

2.1. Honey samples and chemicals

One hundred twenty nine honey samples of various floral origins and colours were analysed. These included: Loco (*Astragalus bisulcatus*), Opoponax-Tree, Alfalfa (*Medicago sativa*), Barberry (*Berberis vulgaris*), Thyme (*Thymus vulgaris*), Argentine thistle (*Eryngium billardieri*), and Dill (*Anethum graveolens dhi*). Samples were obtained directly from bee keepers of several different provinces of Iran. All chemicals and solvents used for analysis were analytical grade with the highest purity available. Folin–Ciocalteu's phenol reagent, gallic acid, sodium carbonate anhydrous, sodium acetate trihydrate, ferrous ammonium sulphate, and ferric chloride hexahydrate were purchased from Merck (Merck KGaA, Darmstadt, Germany). Furthermore, 2,4,6-tripyridyl-s-triazine (TPTZ) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany).

2.2. Colour measurement using Hunter lab and determination of ash content (AC)

Before colour analysis, samples were heated at 50 °C for 10 min to dissolve sugar crystals. Then, they were paved and pureed into 5 cm Petri dishes at 1 cm height. Colour characteristics were measured using the CIE $L^*a^*b^*$ colourimetric method. Honey colour values were determined using a commercial colourimeter (Hunter Lab, color Flex, USA).

Percent ash content was determined for all honey samples based on AOAC (1990).

2.3. Total phenolic content (TPC)

The Folin–Ciocalteu method was utilized to determine the total Phenolic content of the samples (Alvarez-Suarez et al., 2010). Honey solution in distilled water was prepared (10% w/v) and 0.5 ml of this solution was poured into a test tube. Then, 2.5 ml of Folin–Ciocalteu 0.2 N was added and well mixed. After 5 min, 2 ml of 0.7 M sodium carbonate was added. This solution was incubated in dark room at 25 °C for 2 h. Absorbance of the reaction mixture was measured at 760 nm against the sugar analogue using spectrophotometer (Jenway, model 6505). Gallic acid was used as the standard to produce the calibration curve (50–250 mg/L). The total Phenolic content was expressed in mg of Gallic acid equivalents (mg GAE/kg of honey).

2.4. Quantification of antioxidant activity (AA)

Several methods for determining the anti-oxidative activity in honey have been employed. The techniques to evaluate antioxidant capacity are based on colourimetric assays such as DPPH, FRAP, TEAC (ABTS) and microplate fluorescence reader like ORAC assay (Alvarez-Suarez et al., 2009). Antioxidants stabilize free radicals by donating electrons and the antioxidant capacity provided by this mechanism is determined by measuring the reduction capacity of metal ions such as ferric ion (Fe^{3+}): FRAP (Niki, 2011). In this study, FRAP assay has been used for determination of honey antioxidant capacity (ferric reducing/antioxidant power). It is a simple direct test that widely used for assessment of antioxidant activity of various materials including honey (Bertoncelj et al., 2007).

2.4.1. The ferric reducing antioxidant power assay

Benzie and Strain (1996) procedure with minor modification was used for FRAP assay. The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous, coloured form (Fe^{2+}-TPTZ) in the presence of antioxidants. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer, pH 3.6. It was prepared daily and warmed to 37 °C. Aliquots of 400 μl of sample were mixed with 3.6 ml of FRAP reagent and the absorbance of the reaction mixture was measured at 593 nm after incubation at 37 °C for 10 min against the sugar analogue. Ammonium ferrous sulphate (100–1000 μM) was used for the calibration curves and the results were expressed as μmoles of ammonium ferrous sulphate per 100 g of honey (μmol Fe (II)/100 g of honey).

All tests were carried out in triplicate and their averages were reported. PASW statistics software was utilized for statistical analysis.

2.5. Computer vision system (CVS)

The computer vision system used in this work consists of four major components: dark chamber and lighting system, digital camera, computer hardware and software. A $30 \times 30 \times 45$ cm^3 wooden box was prepared to act as the housing. The bottom part of this box (15 cm) was used to form the illuminating system. Furthermore, the upper 30 cm was used to form the dark chamber. A plastic light diffuser sheet which is used to hold the sample separates these two sections from each other. Back-lighting was used for illumination of honey samples to diminish the negative effect of reflectance and to create homogenous light intensity on the honey sample surface. The back-lighting system consisted of a $30 \times 30 \times 15$ cm^3 box with its internal surfaces covered using a reflective sheet for more light scattering in the lighting chamber. LED lamps were placed horizontally in the chamber. A plastic light diffuser was placed on top of the chamber. A Canon 550 D kiss x4 camera was mounted vertically above the back lighting chamber at a distance of 20 cm. The camera was connected to the USB port of a Laptop (Dell, Inspiron 4030, China) provided with a remote capture software (Zoom Browser Ex. Version: 6.7) to view and to capture images from the camera setting controller. The internal surfaces of the wooden box were covered with dark sheets to prevent reflection and external light. The Honey sample was located against the center of the background (Fig. 1a). A standard grey card (10×12 cm^2) with 18% reflectance (Kodak, USA) was used to set