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Analytical Methods

Determination of DPPH free radical scavenging activity: Application of artificial neural networks

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A B S T R A C T

A new computational approach for the determination of 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity (DPPH-RSA) in food is reported, based on the concept of machine learning. Trolox standard was mix with DPPH at different concentrations to produce different colors from purple to yellow. Artificial neural network (ANN) was trained on a typical set of images of the DPPH radical reacting with different levels of Trolox. This allowed the neural network to classify future images of any sample into the correct class of RSA level. The ANN was then able to determine the DPPH-RSA of cinnamon, clove, mung bean, red bean, red rice, brown rice, black rice and tea extract and the results were compared with data obtained using a spectrophotometer. The application of ANN correlated well to the spectrophotometric classical procedure and thus do not require the use of spectrophotometer, and it could be used to obtain semi-quantitative results of DPPH-RSA.

1. Introduction

Oxidation is one of the most important processes in food deterioration as it can affect food safety, color, flavor and texture. Antioxidants prevent the presumed deleterious effects of free radicals in the human body, and prevent the deterioration of fats and other constituents of foodstuffs (Molyneux, 2004). Among the assays that measure radical scavenging capacity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH; Brand-Williams, Cuvelier, & Berset, 1995) assay is one of the most widely used. During this assay, the purple chromogen radical is reduced by antioxidant/reducing compounds (hydrogen-donating antioxidants) to the corresponding pale yellow hydrazine, and the reaction is monitored at 515–520 nm. The reaction is shown in Fig. 1, where AH is a donor molecule, and $A^{\cdot}$ is the free radical produced. In our previous work, a method involved coating the surface of a 96-well microplate with a methanolic DPPH solution and drying it under nitrogen. The DPPH dry reagent array offers advantages compared with the classical solution-based assays: it is rapid, sensitive, cost-effective, facile, uses less reagents, and can be stored for up to 6 months (Musa, Abdullah, Kuswandi, & Hidayat, 2013).

Machine learning is a technique based on computational learning theory, whereby a computational model is built from datasets (e.g. images), rather than dictated by explicit programming instructions. Machine learning might be necessary when it is difficult or impossible to solve a problem using the traditional explicit programming. The aim in machine learning is to use computational intelligent algorithms to train the computer on handling such problems. In machine learning, classification is a common task where a model is constructed to map samples of input data into two or more output classes (Kotsiantis, 2007). The model is first learnt from adequate samples during a training phase, then tested using new samples that the model has not encountered during the learning phase. One of the most popular models used to perform classification is the artificial neural network (ANN; Zhang, 2000), inspired by the brain structure, and used in a vast amount of applications ranging from email filtering to cancer diagnosis (Baxt, 1995; Clark, Koprinska, & Poon, 2003).

ANN was used by Balejko, Nowak, and Balejko (2012) as a tool for predicting rheological features of raw minced meat. Cabrera and Prieto (2010) applied an ANN to the prediction of the antioxidant activity of essential oils. An artificial neural networks for the prediction of antioxidant capacity of cruciferous sprouts was also reported by Bucinski, Zieliński, and Kozłowska (2004); and Cimpoiu, Cristea, Hosu, Sandru, and Seserman (2011) used them to predict the antioxidant activity of some teas. Artificial neural network modelling of the antioxidant activity and phenolic compounds of bananas submitted to different drying treatments was recently reported by Guine et al. (2015).

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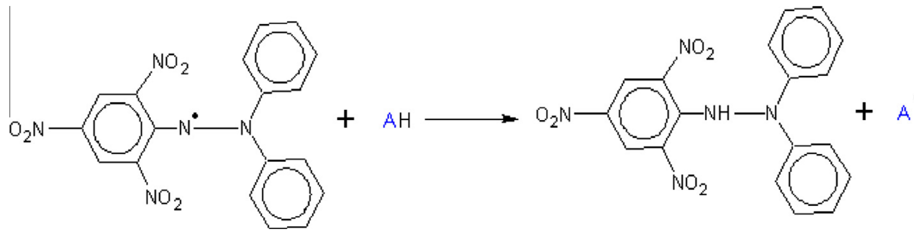

Fig. 1. The reaction of DPPH free radical with antioxidant where AH is donor molecule and A· is free radical produced.

The current procedure of DPPH free radical scavenging activity (DPPH-RSA) determination is based on the measurement of certain properties of light as a function of wavelength using a spectrophotometer. If we take away the details of reflection or transmission at the wavelength level, we are left with a function of visual colors, each of which represents a degree of DPPH-RSA. The levels of colors are distinguishable with the naked eye and by an ordinary optical instrument like a digital camera. The task of measuring DPPH-RSA is thus mapped into a problem of classifying images of the DPPH-RSA, based on their color. This sort of image classification is a typical application in a subfield of computer science known as machine learning (Anderson, Michalski, Carbonell, & Mitchell, 1986; Mitchell, 1999, 2006). Capturing images using digital cameras is an easy, user-friendly task, as is running a software application on a computer or smartphone. No literacy in information technology is required, and no learning curve is involved. And above all, the process is extremely cost-effective compared with the current practice of using spectrophotometers.

Motivated by the above observations, a novel application of machine learning via an ANN model for the determination of DPPH-RSA is presented here. The aim is to train a neural network on a typical set of images representing a mixture of DPPH radical and different concentrations of antioxidant standard. The purpose of training is to teach the ANN how to classify future images of the DPPH-RSA at the correct level. We describe the complete learning process, including the necessary cleaning of the sample images, feature extraction, training and testing the performance of the developed ANN.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and DPPH were purchased from Sigma–Aldrich (USA). Methanol was purchased from Friendemann Schmidt (Germany). All chemicals were of analytical reagent grade.

2.2. Preparation of plant extracts

Cinnamon, clove, mung bean, red bean, red rice, brown rice, black rice and tea were extracted. Briefly, 0.5 g of the plant material was mixed with 10 mL of HCl in aqueous methanol (50%) for 3 min in a 50 mL glass tube and the tube was tightly closed. The mixture was heated at 90 °C for 2 h in a water bath (New Brunswick Scientific, USA). The extract was made up to 20 mL with deionized water, allowed to cool and then filtered through a 0.2 μm nylon filter (Whatman, USA).

2.3. Determination of DPPH free radical scavenging activity

DPPH solution in methanol was added to each of 96 wells of the microtiter plate, and evaporated under nitrogen to form a layer of dried DPPH (30 μg DPPH per well). For machine learning purposes, 300 μL of Trolox standard was added to each well at five different concentrations 0, 25, 33, 42 or 50 μg Trolox per mL. Plates were mixed, covered and incubated in the dark at room temperature for 10 min and then the absorbance was measured at 517 nm using a microplate reader (Spectrostar Nano, BMG Labtech, Australia). The DPPH assay method was reported as radical scavenging activity (RSA%) using the following equation:

$$\mathrm{RSA\%} = [\text{Absorbance of control} - \text{Absorbance of sample}]/[\text{Absorbance of control}] \times 100$$

Plant extracts were used to test the quality of the machine learning program. Plant extracts (300 μL) were added to the DPPH plate at a concentrations of 0, 7, 14, 28, 56 and 83 μg of sample per mL and calculated as described above.

2.4. Machine learning methodology

The machine learning processing steps include image collection, image preprocessing, feature selection, and classification (Fig. 2).

2.5. Image collection

The raw data were images of 96 microtiter plates representing different levels of antioxidant activity against DPPH (Fig. 3a). An eight megapixel back camera of a Samsung Galaxy grand Dous™ smartphone supported by the Android operating system was used.

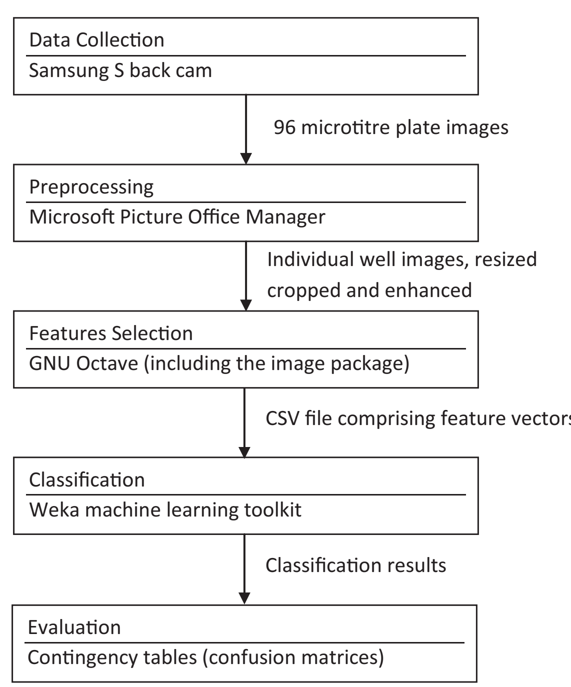

Fig. 2. Block diagram of machine-learning methodology steps.