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Classification of garlic cultivars using an electronic nose

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ABSTRACT

We developed an electronic nose method for classifying garlic cultivars. Each garlic cultivar gave different semiconductor gas sensor array response patterns, which we analysed using two-dimensional principal component analysis (PCA). The method was able to detect the differences between cultivars using a PCA-optimized set of five sensors without any significant loss of performance compared to the original eight sensors. The performance of the electronic nose system was confirmed using two alternative methods, namely (1) the cluster analysis of the genetic relationships between garlic cultivars using amplified fragment length polymorphism (AFLP) markers, and (2) measuring the concentrations of sulfur-containing compounds in each garlic cultivar by gas chromatography-mass spectrometry (GC–MS). Four Thai garlic cultivars were consistently characterized and placed in three groups using the AFLP, GC–MS, and electronic nose methods. These results suggest that garlic cultivars can be classified simply and quickly using a low-cost electronic nose system.

1. Introduction

Garlic (*Allium sativum* L.) is a bulbous plant that is used as a vegetable and for medicinal purposes, being a rich source of carbohydrates and protein, and containing large amounts of sulfur-based substances (Salunkhe and Kadam, 1998). The characteristic flavours of garlic are derived from allicin. When fresh garlic is crushed, alliin, a derivative of the amino acid cysteine, is rapidly converted into allicin by the enzyme alliinase. Allicin and other thiosulfinates are readily oxidized into several volatile compounds (Lanzotti, 2006), some of which (hydrogen sulphide, methyl sulphide, dimethyl sulphide, and allyl sulphide) are claimed to be the main causes of garlic malodours (Tamaki et al., 2008). The pharmacologically active vinyldithiins are also derived from allicin (Sticher, 1991), with approximately 70% of allicin having been found to be transformed into the vinyldithiins 2-vinyl-4H-1, 3-dithiin and 3-vinyl-4H-1,2-dithiin in non-polar organic solvents (Block et al., 1986). The total amount of vinyldithiins produced can, therefore, be considered to be representative of the allicin content in a garlic cultivar.

Some garlic cultivars are famous for their unique flavours and tastes, and classifying garlic cultivars is necessary for their sustainable production and the conservation of plant resources. However, because of their similar morphological characteristics, classifying garlic cultivars from their vegetative anatomy is very difficult. Garlic specimens from different sources have been classified using various methods, such as by comparing their oil contents (Wei, 2001), metal profiling (Smith, 2005), measuring their bioactive compound contents (Gorinstein et al., 2005), classifying their DNA fingerprints (Al-Zahim et al., 1997; Ipek et al., 2003), and using high performance liquid chromatography (Bocchini et al., 2001) or gas chromatography-mass spectrometry (GC–MS; Mazza et al., 1992; Yu et al., 1989). However, these methods are inconvenient because they are complicated, require specialized skills, are labour and time intensive, and are relatively costly. A simple and reliable method for classifying garlic cultivars is therefore needed.

An electronic nose system, which is a simple and fast method of using a sensor array to detect and discriminate between complex odours, has been successfully used to evaluate the odour of garlic (Abbey et al., 2001; Tamaki et al., 2008). The sensor array typically consists of a group of non-specific gas sensors, and an odour is classified from the response pattern for all of the sensors rather the response of any particular one (Li, 2009). Ready-to-use gas sensor

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modules, particularly metal oxide semiconductor ones, have recently become commercially available and have been widely used in sensor arrays for odour measurements (Brattoli et al., 2011). These modules allow portable odour measurement systems to be developed relatively easily and at low cost.

In the study reported here, we developed a low-cost portable electronic nose system to classify garlic cultivars, and evaluated its ability to classify cultivars compared to 'conventional methods', such as amplified fragment length polymorphism (AFLP) and GC–MS. The differences between four garlic cultivars were primarily verified using their AFLP DNA fingerprints, but the different sulfur-containing compounds present in each garlic cultivar were also analysed using GC–MS, and the AFLP and GC–MS results confirmed that the electronic nose system that we developed was effective in classifying the garlic cultivars.

2. Materials and methods

2.1. Garlic samples

Fresh specimens of four garlic cultivars were purchased from local growers in areas of Thailand where garlic is produced in large quantities, namely Uttaradit (UD), Lamphun (LP), and Chiang Mai (CM), which are Northern provinces, and Si Sa Ket (SK), which is in the north-east of Thailand.

2.2. DNA extraction and amplified fragment length polymorphism analysis

Whole genomic DNA was extracted from dried leaves using a modified CTAB method (Doyle, 1990). AFLP analysis was performed following a published method (Vos et al., 1995), with slight modifications. Briefly, 100 ng of genomic DNA was digested with 10 U of *Eco*RI (Fermentas, Ontario, Canada) and 10 U of *Mse*I (Fermentas) restriction enzymes, linked to specific adapters, and the fragments were amplified. The pre-selective amplification reaction was performed using 1 × polymerase chain reaction (PCR) buffer, 2 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates, 0.2 μM *Eco*RI and *Mse*I adapter-directed primers (each possessing a single-selective nucleotide), 0.50 U of *Taq* DNA polymerase (Invitrogen, California, USA), and 2 μL diluted digestion/ligation reactants, in a final reaction volume of 25 μL. The PCR conditions were: 1 min initial denaturation at 94 °C; followed by 25 cycles, each of 30 s denaturation at 94 °C, 30 s primer annealing at 55 °C, and 1 min of primer extension at 72 °C; then a 10 min postcycling extension at 72 °C. The pre-amplification products were diluted 40-fold in 0.1 × Tris–EDTA buffer.

Selective amplification (the second PCR) was carried out using nine selected primer combinations that each had three selective nucleotides. Selective PCR was performed on 3 μL of the diluted pre-amplification reaction mixture described above. The PCR conditions were: 1 min initial denaturation at 94 °C; followed by 35 cycles, each of 30 s denaturation at 94 °C, 30 s primer annealing at 65 °C, which was then reduced by 0.7 °C in each of the next 12 cycles and then maintained at 56 °C for the remaining cycles, and 1 min primer extension at 72 °C; then a 10 min postcycling extension at 72 °C. The PCR amplification products were separated on 7% denaturing polyacrylamide silver stained gels (Benbouza et al., 2006). Cleared AFLP bands were scored manually, with a value of 1(0) being used for the presence (absence) of bands. Each garlic cultivar was analyzed three times (Table 1). Genetic similarities were estimated using Jaccard's similarity coefficient (Jaccard, 1908). The phylogenetic tree was constructed using the unweighted pair-group method with arithmetic averages (UPGMA) using NTSYS-Pc computer software, version 2.2 k (Rohlf, 2005).

Table 1
Sample names and origins of the garlic cultivars used in the AFLP analysis.

Origin	Number of samples	Sample names
Uttaradit	3	UD1, UD2, UD3
Lamphun	3	LP1, LP2, LP3
Chiang Mai	3	CM1, CM2, CM3
Si Sa Ket	3	SK1, SK2, SK3

2.3. GC–MS analysis

Three raw garlic samples were extracted for each garlic cultivar. Each sample was peeled, crushed with a spoon, and a 1 g aliquot placed in a screw cap vial and allowed to stand at room temperature for 5 min. The crushed garlic was then extracted with 5.0 mL of dichloromethane, and the extract was filtered through a 0.22 μm nylon membrane filter. The filtrate was analyzed twice by GC–MS.

GC separation was achieved using a 5% phenyl, 95% dimethyl polysiloxane (DB-5) fused-silica capillary column (30 m long, 0.32 mm i.d., 0.25 μm film; Agilent Technologies, California, USA). The instrument was a 6890 N gas chromatograph with a 5973 mass selective detector (Agilent Technologies). The GC temperature programme started at 50 °C, increased at 5 °C min^{-1} to 150 °C, held there for 3 min, then increased at 30 °C min^{-1} to 250 °C, and held there for 1 min. The carrier gas was He, flowing at 51 cm s^{-1}, and the electron ionization mode was used, with electron energy of 70 eV.

2.4. Electronic nose system

The electronic nose system that was developed to measure and classify garlic odours is shown schematically in Fig. 1(a). Eight gas sensors were arranged in the lid of a 2.4 L chamber to form the sensor array. Sensors S1 and S2 were MQ-2 and MQ-135 (Henan Hanwei Electronics, Henan, China), respectively, and the other sensors were TGS822 (S3), TGS826 (S4), TGS2600 (S5), TGS2602 (S6), TGS2611 (S7), and TGS2620 (S8) (Figaro Engineering, Osaka, Japan). The sensing materials in all of the sensors were metal oxide semiconductors. The measuring circuit of each sensor was also shown in Fig. 1(b). A 5 V common power supply is used for the heater voltage (V_h) and the circuit voltage (V_{cc}). The sensor response of sensor *N*th is obtained by measurement of voltage (V_{SN}) across a load resistor (R_{SN}) as shown in Fig. 1(b). In order to achieve appropriate sensor response, the load resistor with selected resistance was connected in series with each sensor. The values of the resistors R_{S1}–R_{S8} are set to 3.33 kΩ, 6.79 kΩ, 6.89 kΩ, 10.98 kΩ, 3.16 kΩ, 2.97 kΩ, 7.95 kΩ, and 1.86 kΩ respectively, in order to equalize all baseline voltages to 0.5 V. The resistance of each resistor was fixed in all measurement to avoid biasing in the sensor response. The output responses (voltages) of the sensors were converted into digital signals using a multichannel analogue–digital (A/D) converter (USB6009; National Instrument, Texas, USA) at 14-bit resolution and sent to a personal computer for processing. A pump and solenoid valves were used to purge the odour from the measurement chamber after each measurement was complete, to keep the sensor baseline consistent.

To take a measurement, 1.5 g of crushed raw garlic was placed on a petri dish and placed inside the chamber, at room temperature. The odour from the crushed garlic sample was measured by the sensor array for 600 s, the data from the sensor array being collected every second. The measurement process was automatically controlled by a program we developed using LabView (National Instrument). Samples of each garlic cultivar were prepared and measured five times. The sensor responses obtained were analyzed using principal component analysis (PCA), to classify the patterns associated with each garlic cultivar.