Non-destructive flavour evaluation of red onion (*Allium cepa* L.)
Ecotypes: An electronic-nose-based approach

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**A R T I C L E  I N F O**

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**A B S T R A C T**

This work reports preliminary results on the potential of a metal oxide sensor (MOS)-based electronic nose, as a non-destructive method to discriminate three “Tropea Red Onion” PGI ecotypes (TrT, TrMC and TrA) from each other and the common red onion (RO), which is usually used to counterfeite. The signals from the sensor array were processed using a canonical discriminant function analysis (DFA) pattern recognition technique. The DFA on onion samples showed a clear separation among the four onion groups with an overall correct classification rate (CR) of 97.5%.

Onion flavour is closely linked to pungecy and thus to the pyruvic acid content. The e-nose analysis results are in good agreement with pyruvic acid analysis. This work demonstrated that artificial olfactory systems have potential for use as an innovative, rapid and specific non-destructive technique, and may provide a method to protect food products against counterfeiting.

**1. Introduction**

*Allium cepa* L. is a species of great economic importance, which is widely cultivated all over the world. The health benefits of onion consumption include protection against cancer, coronary heart disease, diabetes and ageing. These effects have been mainly attributed to flavonoids, vitamins and organosulphur compounds (Goldman, Kopelberg, Debaene, & Schwartz, 1996; Kumari, Matthew, & Augusti, 1995). Although they possess significant nutritional value, onions are primarily consumed for their unique flavour and for their ability to enhance the taste of foods (Kopsell & Randle, 1997; Rodrigues et al., 2003).

Onions are widely cultivated throughout the Mediterranean basin and in Italy. The “Tropea Red Onion” (TrO), a sweet red onion characterised by its elongated bulb and almost white flesh, is cultivated, in three different ecotypes, only in the Tyrrehnian coastal areas of Calabria region (Southern Italy). In March 2008, the European Union registered the Protected Geographical Indication (PGI) certification for the onions produced in this particular area (Commission Regulation (EC) No. 284/2008). The ‘Cipolla Rossa di Tropea – Calabria’ PGI denotes bulbs of the species *A. cepa* exclusively from the local ecotypes. These have a characteristic shape according to time of production; ‘Tonda Piatta’, the early crop; ‘Mezza Campana’, the mid to early crop; ‘Allungata’, the late crop. TrO, for its tenderness, crispness and sweetness is a highly regarded Italian horticultural product and one of the most counterfeited.

Sensory evaluation of onion flavour is closely linked to its pungency, which shows a close correlation with its pyruvate content (Schwimmer & Weston, 1961). The pungent flavour of onions is produced by hydrolysis of precusor compounds, such as S-alk(en)yl-L-cysteine sulfoxides (Lancaster, Shaw, Joyce, McCallum, & McManus, 2000), when the cells are mechanically ruptured. The hydrolysis reaction is catalysed by alliinase and is completed within 6 min (Schwimmer & Weston, 1961). This reaction produces thioipropanol S-oxide (a lacrymator), ammonia, sulfur volatiles and pyruvic acid (Fig. 1; Block, 1992). Pyruvic acid concentration (μmol/g FW) in macerated onion tissue is used as a quality assurance indicator of pungency (Abayomi, Terry, White, & Warner, 2006; Pineda, Lué-Merú, Rivas, et al., 2004). Pyruvic acid exists universally in the plant tissues as part of an intermediate metabolism (Goodwin & Mercer, 1983). For this reason, the background levels of pyruvic acid or control (Pc) need to be subtracted from the total pyruvic acid (Pt) concentrations to calculate the enzymatically produced pyruvate (Pc). Background pyruvic acid can be measured after the alliinase is deactivated. The deactivation of alliinase has been achieved by heating onion tissues in a
microwave oven (Schwimmer & Weston, 1961; Yoo, Pike, & Hamilton, 1995) or by homogenising with trichloroacetic acid (Randell & Bussard, 1993). Since the PC level can substantially change the Pc concentration, its accurate measurement is critical in measuring onion pungency (Boyhan, Schmidt, Woods, Himelrick, & Randle, 1999; Thomas, Parkin, & Simon, 1992; Yoo & Pike, 2001). Since the PC concentration and human organoleptic profiling panels. These methods are expensive not only in terms of time, but are also inaccurate because of a lack of either sensitivity or quantitative information. In this paper an investigation was carried out to determine the flavour of different onion samples using an electronic nose (e-nose) and thus to explore the possibility of replacing existing analytical methods.

The e-nose is a relatively novel device used for volatile sensing. The first pioneering studies about the concept of an artificial nose system able to measure odours was reported in 1982 by Persaud and Dodd of the University of Warwick, Coventry, UK (Cho et al., 2010). The e-nose has been designed for automated detection and recognition of odours, vapours and gases (Cho et al., 2010). It does not separate the volatile fraction of the matrix into its constituents but supplies a global evaluation of aroma, mimicking the human olfactory system with instrumental objectivity.

Electronic nose technology has been successfully used to discriminate quality and flavour of various products, including tomatoes, citrus, spices and onions (Abbey & Joyce, 2007; Russo, Serra, Suraci, & Postorino, 2012). The technology may also be used to assess quality of stored grain, fish, drugs, drinks and food spoilage (Abbey, Aked, & Joyce, 2001).

The e-nose used in this work comprises an array of semiconductor gas sensors (MOS), each of which has an electrical resistance that has partial sensitivity to the headspace of onion. The signals from the sensor array are then conditioned by suitable interface circuitry, resulting in an onion data-set. The data were processed using a canonical discriminant function analysis (DFA) pattern recognition tool. DFA has been used extensively to perform pattern recognition and it has been reported to produce good performance for the classification of foodstuffs (Russo et al., 2012). DFA was used to check the capability of the e-nose system in assigning onion samples to a specific group.

Finally, the aim of this study was to discriminate flavour and aroma characteristics of the early, middle and late crops of Tropea Red Onion and also distinguish between them and the common red onion by the use of an electronic nose. Studies on different ecotypes, to the best of our knowledge, have not been carried out before. The analysis of pyruvic acid by HPLC with UV detection was also carried out.

2. Materials and methods

2.1. Plant materials

In 2011 the three local ecotypes of Tropea red onion and one red onion cultivar usually sold as Tropea, were analysed and compared (Table 1). All onion types were grown in a field in IGP district and on the same farm, so that the evaluation was not influenced by environmental factors and/or cultural practices. Three different samples were collected for each type, consisting of five onions. Each analysis was performed in triplicate for each sampling.

2.2. Electronic nose

An electronic nose (ISENose 2000, Labservice Analytica, Bologna, Italy) was used to discriminate aroma fingerprints of onion ecotypes according to the method of Abbey et al. (2001). The edible parts of the onions were homogenised in an Ultra-Turrax system (T50 basic, IKA Werke, Staufen, Germany) at room temperature. The slurry was filtered after 20 min and 20 ml of the filtrate were transferred into a 250-ml flask.

Twenty millilitres of 5% trichloroacetic acid (TCA; Sigma Chemical Co., Milano, Italy) was used to terminate alliinase activity. The mixture was agitated vigorously and allowed to stand for 30 min. Deionised water (10 ml) was added to 10 ml of filtrate/TCA solution and mixed. One millilitre of this diluted solution was put into a 25-ml vial, and equilibrated for 10 min at 25 °C to allow the development of headspace before e-nose analysis. The instrument, equipped with 12 metal oxide semiconductor (MOS) sensors, was used to generate a pattern for the compounds present in the headspace of the extracted red onion samples. The operating conditions in the acquisition phase were: baseline correction 10 s; injection time 10 s; sampling 180 s; delay 300 s; purge 60 s; carrier chromatographic air (80% N2, 20% O2) flow 300 ml/min; vial volume 25 ml.

2.3. Pyruvic acid extraction procedure and HPLC analysis

The quantification of the pyruvic acid content in the onion was conducted using a modified method of Yoo and Pike (2001). The neck, base and central leaf tissues (about 5 cm long) were removed and the remaining fresh scales were cut into 1–2 cm squares using

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Red Onion ecotypes.</th>
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</thead>
<tbody>
<tr>
<td>ID</td>
<td>Sample</td>
</tr>
<tr>
<td>TrT</td>
<td>Tropea Ecotype &quot;Tonda&quot;</td>
</tr>
<tr>
<td>TrMC</td>
<td>Tropea Ecotype &quot;Mezza Campana&quot;</td>
</tr>
<tr>
<td>TrA</td>
<td>Tropea Ecotype &quot;Allungata&quot;</td>
</tr>
<tr>
<td>RO</td>
<td>Red Onion</td>
</tr>
</tbody>
</table>
a razor blade. The cut pieces (~10 kg) were mixed thoroughly, rinsed with water and drained to remove the soil residues. As a control to measure the background level of pyruvic acid ($P_b$), 100 g tissue were placed in a plastic bag (17.5 cm × 21 cm). The samples were heated in a microwave oven (AFM 442 Ultimys Duo Grill; Moulinez, Italy) for 3 min at 900 W to deactivate the alli-nase. After microwaving, the sample was placed in a bag under ice to stop the cooking. The tissue was blended in an Ultra-Turrax system for 2 min and the juice was collected through centrifugation (Eppendorf 5804R; Milano, Italy) and filtration with a syringe filter. To measure total (background and alli-nase-produced) pyruvic acid content ($P_t$), 100 g tissue were blended in an Ultra–Turrax system for 2 min. The homogenised mixture was macerated for 30 min at room temperature and juice was collected through centrifugation and filtration with syringe filter.

Chromatographic analysis on onion extracts was carried out using an HPLC (LC-10AD system; Shimadzu, Kyoto, Japan) with diode array detector. Each sample extract was injected in duplicate on a 300 × 7.8 mm i.d. Suplecogel C610H (Sigma–Aldrich, St Louis, MO) and eluted using 0.3 ml/l H$_2$SO$_4$ at a flow rate of 0.5 ml/min. The eluate was monitored at 210 nm. Standards were prepared with pyruvic acid, purchased from Sigma Chemical Co. (Milano, Italy). Stock and working standards were prepared in HPLC-grade methanol.

Enzymatically produced (from alli-nase) pyruvate in each sample was calculated from the difference of the two determinations. All analyses were run in triplicate.

### 2.4. Data analysis

The pyruvic acid content in onion extracts was identified by its retention time characteristics. The linearity of the detector response for the prepared standards was assessed by means of linear regression, based on the amounts of each standard and the area of the corresponding peak on the chromatogram. The limit of detection (LOD) and quantification (LOQ) were determined by calculation of the signal-to-noise ratio. Signal-to-noise ratios of approximately 3:1 and 10:1 were used for estimating the detection limit and quantification limit, respectively. LOD and LOQ values of pyruvic acid were 0.029 and 0.088 mmol/kg respectively; $r^2$ Values of the compounds were higher than 0.999, thus confirming the linearity of the method.

The precision of injection was demonstrated by replicate injections of the standard solution and the relative standard deviation (RSD) of peak area was 0.38% ($n = 8$).

The sensors’ responses were evaluated using discriminant function analysis (DFA) by IseNose OCS software (version DT 01837 Rev. 02A, ISENose 2000; Labservice Analytica, Bologna, Italy).

### 3. Results and discussion

The sensor responses were evaluated using Discriminant Function Analysis (DFA). The four samples (three local ecotypes and one common red onion) were chosen as grouping variables and sensors outputs were used as independent variables. A comprehensive database (500 data points) was developed to increase the reliability of the analysis. Four different sets of e-nose data clusters for onion headspace volatiles were identified in the DFA analysis (Fig. 2). Three clusters matched the three ecotypes of Tropea red onion (TrT red, TrA azure and TrMC blue) and one cluster matched the red onion (RO green).

E-nose data set clusters for headspace volatiles of three types of Tropea red onion and common red onion are shown in Fig. 2. The e-nose discriminates between the three Tropea Red Onion ecotypes and between them and common RO (Fig. 2). Discrimination is considered acceptable when it reaches values up to 80%; values around 85% are considered a sign of good discrimination, while values up to 90% indicated excellent discrimination. The DFA on onion samples showed a clear separation among the four onion groups, with an overall correct classification rate (CR) of 97.5%. These results prove that the e-nose is capable of discriminating between the flavours of onions.

The e-nose results were compared with pyruvic acid content estimates. It is generally accepted that there is a high correlation between levels of enzymatically-produced pyruvate and perception of pungency (Schwimmer & Weston, 1961). The amount of pyruvic acid produced due to alli-nase activity following tissue damage was evaluated in all samples. The theoretical 1:1 M ratio between pyruvate and its flavour precursors (alk(en)lycysteinesulphoxides) (Yoo & Pike, 2001) suggested that the HPLC analysis of pyruvic acid content may represent a useful estimation of onion pungency (Bacon et al., 1999).

Previous study reports considerable variation in background pyruvic acid concentrations. Schwimmer and Weston (1961) reported background levels of 2.1–4.1 µmol/ml from White Grano, Southport Red Globe, and five other cultivars. Randle and Bussard (1993) reported 0.1–1.0 µmol/ml pyruvate from 16 short-day onion cultivars, using the original Schwimmer and Weston method. Yoo et al. (1995) reported less than 0.5 µmol/ml using undiluted onion juice and suggested that measurement of the background level could be disregarded. Luo and Ewart (1995) reported background levels of 0.79 µmol/ml. The background pyruvic acid concentrations in our analysed samples showed levels of 0.22 ± 0.12 to 0.30 ± 0.11 µmol/g, lower than previously reported.

The sweet onion industry in USA classifies onions on the basis of pungency as low pungency/sweet (0–3 µmol pyruvic acid/g FW), medium pungency (3–7 µmol pyruvic acid/g FW), and high pungency (above 7 µmol pyruvic acid/g FW) (Dhumal, Datir, & Pandey, 2007). Based on this classification, the sample RO can be considered as a medium pungent cultivar (3.79 ± 0.86 µmol/g) and all the three Tropea ecotypes as low pungency cultivars (Table 2). Of the three Tropea ecotypes, TrA shows the highest concentration of pyruvic acid (2.57 ± 0.81 µmol/g), TrMC shows a middle value (2.45 ± 0.30 µmol/g) while TrT shows the lowest concentration.
Table 2

<table>
<thead>
<tr>
<th>ID</th>
<th>PC (mol/g fw)</th>
<th>PT (mol/g fw)</th>
<th>PE (mol/g fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrT</td>
<td>2.26 ± 0.61</td>
<td>0.30 ± 0.11</td>
<td>1.96 ± 0.06</td>
</tr>
<tr>
<td>TrA</td>
<td>2.57 ± 0.81</td>
<td>0.26 ± 0.16</td>
<td>2.31 ± 0.81</td>
</tr>
<tr>
<td>TrMC</td>
<td>2.45 ± 0.30</td>
<td>0.22 ± 0.12</td>
<td>2.23 ± 0.17</td>
</tr>
<tr>
<td>RO</td>
<td>3.79 ± 0.86</td>
<td>0.27 ± 0.12</td>
<td>3.52 ± 0.37</td>
</tr>
</tbody>
</table>

(2.26 ± 0.61 mol/g). The latter results are in agreement with data obtained by the e-nose analysis and with those reported by consumers identifying the ecotype “Tonda” (TrT) as the sweetest. This work showed that the e-nose can discriminate the Tropea red onion from other red onion and the different ecotypes of Tropea Red Onion with respect to their flavour and pungency.

4. Conclusion

It has been shown that over 80% of onion pungency depends on genetic factors, while the remaining part is related to external factors, such as the habitat and cultivation practices (Yoo et al., 2006). Therefore the choice of cultivars appears to be a very important factor in the production of onions with a low level of pungency, while the habitat also influences the organoleptic characteristics.

Further studies are in progress to determine which onion head-space volatile chemicals interact with the e-nose sensors, in order to allow a clear correlation between onion quality and e-nose data sets.

Acknowledgement

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