

# Multielement Fingerprinting as a Tool in Origin Authentication of PGI Food Products: Tropea Red Onion

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**S** Supporting Information

**ABSTRACT:** Tropea red onion (*Allium cepa* L. var. Tropea) is among the most highly appreciated Italian products. It is cultivated in specific areas of Calabria and, due to its characteristics, was recently awarded with the protected geographical indications (PGI) certification from the European Union. A reliable classification of onion samples in groups corresponding to “Tropea” and “non-Tropea” categories is now available to the producers. This important goal has been achieved through the evaluation of three supervised chemometric approaches. Onion samples with PGI brand (120) and onion samples not cultivated following the production regulations (80) were digested by a closed-vessel microwave oven system. ICP-MS equipped with a dynamic reaction cell was used to determine the concentrations of 25 elements (Al, Ba, Ca, Cd, Ce, Cr, Dy, Eu, Fe, Ga, Gd, Ho, La, Mg, Mn, Na, Nd, Ni, Pr, Rb, Sm, Sr, Tl, Y, and Zn). The multielement fingerprint was processed using linear discriminant analysis (LDA) (standard and stepwise), soft independent modeling of class analogy (SIMCA), and back-propagation artificial neural network (BP-ANN). The cross-validation procedure has shown good results in terms of the prediction ability for all of the chemometric models: standard LDA, 94.0%; stepwise LDA, 94.5%; SIMCA, 95.5%; and BP-ANN, 91.5%.

**KEYWORDS:** Tropea red onion, trace elements, ICP-MS, authenticity, chemometric analysis, protected geographical indications

## INTRODUCTION

In 2002 the European Union (EU) approved a council regulation about principles and requirements of food law within the Member States<sup>1</sup> concerning protection of consumer health and functioning of the EU internal market. The concept of traceability was introduced complementing European Economic Community (EEC) regulation 2081/92,<sup>2</sup> in which two separate classes of protected geographical name had been defined, that is, protected designations of origin (PDO) and protected geographical indications (PGI). PDO is a brand used for foodstuffs with a strong regional identity that are produced, processed, and prepared in a specific geographical area using particular techniques, whereas PGI concerns agricultural products and foodstuffs closely linked to a geographical area in at least one of the stages of production, preparation, or processing. In any case, the PGI product has to grow in the region that is designated in its name, and it must have a reputation that can be attributed to its geographical origin. The new rules aim at protecting products against fraud and imitation and at protecting consumers by providing them with information on quality, organoleptic, and nutritional characteristics of the foodstuff. The drawback of all the proposed new strategies in the issued food directives is often represented by the lack of any scientifically based reliable protocol to assess what is claimed in the PDO and PGI labeling.

Onion (*Allium cepa* L.) is one of the oldest cultivated plants, and it is now used both as a food and for medical purposes. In fact, onion is a rich source of a number of phytonutrients, such as flavonoids, fructo-oligosaccharides, thiosulfonates, and other sulfur compounds, which make it an important food of the Mediterranean diet.

The Tropea red onion is a typical Italian variety, cultivated in specific areas of Calabria. This cultivar, characterized by both

white and purple flowers, is known for its distinctive red and sweet bulb (lengthened or oval).<sup>3</sup> Due to its characteristics, it was awarded with PGI certification by the European Union as “Cipolla Rossa di Tropea Calabria”.<sup>4</sup> Analysis of the methanolic extract from the bulbs of Tropea onions revealed the presence of high concentrations of flavonoids, which continue to attract attention as potentially useful agents with implications for inflammation, cardiovascular diseases, and cancer.<sup>5,6</sup> With respect to other onion cultivars, the variety Tropea seems to have peculiar nutritional properties such as a relatively high content of delphinidin derivatives (about 30% of the total anthocyanin content), the presence of petunidin derivatives, and the highest amount of free quercetin (557.8 mg kg<sup>-1</sup> in fresh bulbs).<sup>7</sup> Due to its special characteristics, the Tropea red onion is a product known throughout the world, important for the local and national economies. On the other hand, the Tropea red onion is subject to food fraud. In 2008 it was estimated that, against a production of Tropea red onion in Calabria of about 20,000 tons, the red onions labeled with the PGI brand were over 100,000 tons. This discrepancy can be evidently explained by an importation of onion fraudulently labeled with the PGI brand from abroad.<sup>8,9</sup> Identification of the geographical origin and authenticity of food products represents an important goal to ensure organoleptic and nutritional characteristics to consumers and to prevent unfair competition that can eventually damage the whole agricultural sector. Since early 2000, several papers have been published and a great number of different analytical techniques and parameters have been

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evaluated for geographical origin authentication purpose.<sup>10–13</sup> Among these, mono- and multielemental techniques have been successfully employed in food authentication.<sup>14,15</sup> Recently, we have used multielement profiling for the geographical characterization of olive oil, tomato, and tomato paste and for the detection of buffalo milk adulteration.<sup>16–18</sup>

Onions have been the subject of numerous works, some of which concerned the discrimination of their production area. Ariyama et al. published some important papers in the food forensic field concerning onions and Welsh onions. The elemental concentration profile evaluated by flame atomic absorption spectroscopy (FAAS), inductively coupled plasma emission spectroscopy (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) was applied to Welsh onion samples from Japan and China for the determination of their geographical origin.<sup>19,20</sup> Subsequently, a similar method was successfully applied by the same research group to discriminate the Japanese onions from those from abroad.<sup>21</sup> These studies demonstrated also that the differences in element content by fertilization were smaller than those between production zones when judged from an overview of numerous elements.<sup>22</sup> Some studies have concerned the development and optimization of analytical methods for the determination of major and trace elements in different onion cultivars for comparative investigation between organic and conventional agriculture crops.<sup>23–25</sup> Recently, instrumental neutron activation analysis (INAA) and  $k_0$ -based prompt  $\gamma$ -ray analysis ( $k_0$ -PGA) were used in producing district determinations. The use of these nondestructive analytical techniques avoided the process of acid digestion of onion samples.<sup>26</sup> Infrared spectroscopy, another important nondestructive analytical method, was also used for the determination of quercetins in onions<sup>27</sup> and for authentication purposes.<sup>28</sup>

Other works have been focused on differentiation of cultivars. Quantitative analysis of mineral content carried out by inductively coupled plasma atomic emission spectroscopy (ICP-AES)<sup>29</sup> and flame atomic absorption spectrometry (FAAS)<sup>30</sup> and sensorial analysis carried out by electronic tongue,<sup>31</sup> coupled with proper chemometrics approaches, were used with this aim.

In this work, the capability of multielement profiling to act as marker for the classification of Tropea red onion was evaluated. Twenty-five elements (including 9 lanthanides) were determined by ICP-MS in 120 samples grown according to the production regulations of the Consorzio di tutela della Cipolla Rossa di Tropea (Consortium for the protection of Tropea Red Onion) and 80 onion samples from fields not belonging to the cultivation areas specified in the production regulations. Three supervised pattern recognition chemometric procedures, linear discriminant analysis (LDA), soft independent model class analogy (SIMCA), and artificial neural networks (ANNs), were applied to build models able to discriminate between PGI Tropea and non-Tropea samples. Cross-validation was used to check the reliability of the chemometric models in terms of classification ability.

## MATERIALS AND METHODS

**Chemicals and Instrumentation.** The reagents used for mineralization, HNO<sub>3</sub> (65%) and H<sub>2</sub>O<sub>2</sub> (30%), were Suprapur (Merck, Darmstadt, Germany). All other reagents used for analysis were of analytical reagent grade (Merck). Two multielement solutions of Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, V, U, and Zn (100 mg/L, Merck) and Ce, Dy, Er, Eu,

Gd, Ho, La, Lu, Nd, Pr, Sm, Sc, Tb, Th, Tm, Y, and Yb (10 mg/L, Perkin-Elmer) were used to prepare the calibration standards. Aqueous solutions were prepared using ultrapure water, with a resistivity of 18.2 M $\Omega$  cm, obtained from a Milli-Q plus system (Millipore, Bedford, MA). All glassware, polyethylene flasks, and tubes involved in sample preparation and measurement were cleaned with nitric acid (2%, v/v) overnight, rinsed with ultrapure water, and dried.

An Anton Paar Multiwave 3000 with programmable power control (maximum power 1400 W) and rotor XF100 (operating pressure up to 120 bar maximum; operating temperature, 260 °C maximum; construction material, PTFE-TFM for the liner) was used for the microwave digestion of the samples.

The determination of the elements of interest was carried out utilizing an Elan DRC-e ICP-MS instrument (Perkin-Elmer SCIEX, Canada) equipped with a dynamic reaction cell for suppressing or reducing polyatomic interferences operating with CH<sub>4</sub> (99.996% purity) as reaction gas.

The sample delivery system consisted of a Perkin-Elmer autosampler model AS-93 Plus with a peristaltic pump and a cross-flow nebulizer with a Scott type spray chamber. Samples were introduced by means of a quartz nebulizer. The ICP torch was a standard torch (Fassel type torch) with a platinum injector. A solution containing Rh, Mg, Pb, Ba, and Ce (10  $\mu$ g/L) was used to optimize the instrument in terms of sensitivity, resolution, and mass calibration. The <sup>140</sup>Ce<sup>16</sup>O<sup>+</sup>/<sup>140</sup>Ce<sup>+</sup> and <sup>137</sup>Ba<sup>2+</sup>/<sup>137</sup>Ba<sup>+</sup> ratios were used to check, respectively, the level of oxide ions in the plasma and the level of doubly charged ion that could interfere in the determination of some elements. Moreover, instrumental parameters such as rf power and carrier gas flow were optimized. To assess the accuracy of the method, certified reference material NCS ZC85006 Tomato (China National Analysis Center for Iron and Steel 2000) was submitted to the whole analytical process.

**Sampling.** Samples were provided by the Consortium for the Protection of Tropea Red Onion and were harvested during the crop season 2009 from different fields belonging to the municipalities of Capo Vaticano, Amantea, Nocera Terinese, and Briatico (Figure 1) and immediately stored at –20 °C. Non-Tropea onion samples came from three Italian regions (Campania, Sicilia, and Piemonte) and The Netherlands (Table 1).

**Analytical Procedure.** Ten onions were randomly chosen for each harvest lot. For each onion, outer tunic, leaves, and basal plate with roots were discarded. Only the bulb was homogenized at 25 °C using an electric mixer. An aliquot of onion sample (2 g) was directly weighed into the liner of the microwave system. The digestion was performed by adding 3 mL of HNO<sub>3</sub> and 1 mL of H<sub>2</sub>O<sub>2</sub> to each sample. The operating conditions used for the microwave digestion system are shown in Table 2. After digestion, the extracts were quantitatively transferred to a graduated polypropylene test tube and diluted with ultrapure water to 50 mL. Ten grams of homogenized sample was dried at 80 °C to constant weight to obtain the moisture content for each harvested onion batch.

The analytical batch consisted of a set of calibration standards, which were analyzed at the beginning of the run, samples, a minimum of three procedural blanks, and one procedural blank spiked with a solution containing the elements of interest. A midrange calibration standard was measured at the end of each analytical run, to assess instrumental drift throughout the run. An eight-point calibration curve covering the range of 0.1–2000  $\mu$ g/L was used for quantitative analysis. Standard solutions were prepared by diluting the multielement solutions cited under Chemicals and Instrumentation.

**Statistical Analysis.** Classification was carried out by three multivariate chemometric techniques: artificial neural network (ANN), linear discriminant analysis (LDA), and soft independent modeling of class analogy (SIMCA). ANN and LDA were performed by Statistica 7.1 (StatSoft 2005 edition), and SIMCA was executed by V-Parvus 84



**Figure 1.** Calabria map. In black is spotlighted the cultivation areas of Tropea red Onion.

**Table 1.** Number of Tropea and Non-Tropea Onion Samples

cultivation zone	region/state	harvest time	no. of samples
Capo Vaticano	Calabria	May 2009	20
Amantea	Calabria	May 2009	20
Nocera Terinese	Calabria	June 2009	40
Briatico	Calabria	July 2009	40
Agrigento	Sicily	July 2009	20
Salerno	Campania	June 2009	20
	Piedmont	June 2009	20
	The Netherlands	June 2009	20

2004.<sup>32</sup> Before ANN construction, principal component analysis (PCA) was performed by the Statistica 7.1 statistical package.

## RESULTS AND DISCUSSION

**Analytical Performances.** Digestion of onion samples using a microwave oven system for multielement determination is an issue already addressed in the literature. Bibak et al. proposed a closed vessel mineralization using a pressure program.<sup>23</sup> In a geographical origin investigation study of Japanese onions, Ariyama et al. proposed a microwave sample digestion accomplished using a closed vessel system with temperature program followed by heating on a hot plate to dry the samples.<sup>21</sup> In this work, we have chosen a simpler and more rapid mineralization method, already used for selenium speciation study in onions,<sup>33</sup> which consists of a four-step power program for a closed vessel microwave oven (Table 2). The mineralization mixture was constituted by 3 mL of HNO<sub>3</sub> and 1 mL of H<sub>2</sub>O<sub>2</sub>. Initially, it was decided to monitor a great number of elements including the

**Table 2.** Operating Conditions Used for the Microwave Digestion System

step	power (W)	hold (min)
1	100	5
2	600	5
3	1000	10
4	0	15

rare earth metals because these elements can play an important role in applications of food authentication.<sup>34,35</sup>

The 46 elements investigated were <sup>7</sup>Li, <sup>9</sup>Be, <sup>23</sup>Na, <sup>24</sup>Mg, <sup>27</sup>Al, <sup>39</sup>K, <sup>44</sup>Ca, <sup>45</sup>Sc, <sup>51</sup>V, <sup>52</sup>Cr and <sup>53</sup>Cr, <sup>55</sup>Mn, <sup>54</sup>Fe and <sup>56</sup>Fe, <sup>58</sup>Ni and <sup>60</sup>Ni, <sup>59</sup>Co, <sup>63</sup>Cu, <sup>64</sup>Zn and <sup>66</sup>Zn, <sup>69</sup>Ga, <sup>75</sup>As, <sup>82</sup>Se, <sup>85</sup>Rb, <sup>88</sup>Sr, <sup>89</sup>Y, <sup>107</sup>Ag, <sup>114</sup>Cd, <sup>115</sup>In, <sup>133</sup>Cs, <sup>138</sup>Ba, <sup>139</sup>La, <sup>140</sup>Ce, <sup>141</sup>Pr, <sup>142</sup>Nd, <sup>152</sup>Sm, <sup>153</sup>Eu, <sup>158</sup>Gd, <sup>159</sup>Tb, <sup>164</sup>Dy, <sup>165</sup>Ho, <sup>166</sup>Er, <sup>169</sup>Tm, <sup>174</sup>Yb, <sup>175</sup>Lu, <sup>205</sup>Tl, <sup>208</sup>Pb, <sup>209</sup>Bi, <sup>232</sup>Th, and <sup>238</sup>U. The determination of some of these elements by ICP-MS is known to suffer from polyatomic isobaric interferences. The dynamic reaction cell (DRC) is proved to be an effective method for relieving such isobaric interferences. It is pressurized with an appropriate gas, and elimination or reduction of polyatomic interferences takes place through the interaction of the reaction gas with the interfering polyatomic species in the incoming ion beam. The formation of new interferences avoids eliminating unwanted reaction byproducts, which could otherwise react, through the appropriate use of the rf value in the reaction cell. Sc, Cr, Fe, Ni, Zn, Se, and Eu have been monitored in both modes (standard and DRC modes) using methane (99.996% purity) as reaction gas. According to the polyatomic isobaric interferences for these elements (Table 3), the optimization of the CH<sub>4</sub> flow rate and



**Table 3. Some of the Most Abundant Polyatomic Interferences of the Isotopes Monitored in DRC Mode**

isotope	polyatomic isobaric interferences
<sup>45</sup> Sc	<sup>13</sup> C <sup>16</sup> O <sub>2</sub> <sup>+</sup> , <sup>29</sup> Si <sup>16</sup> O <sup>+</sup>
<sup>52</sup> Cr	<sup>36</sup> Ar <sup>16</sup> O <sup>+</sup> , SO <sup>+</sup> , <sup>38</sup> Ar <sup>14</sup> N <sup>+</sup> , <sup>35</sup> Cl <sup>17</sup> O <sup>+</sup> , <sup>40</sup> Ar <sup>12</sup> C <sup>+</sup> , <sup>1</sup> H <sup>35</sup> Cl <sup>16</sup> O <sup>+</sup>
<sup>54</sup> Fe	<sup>54</sup> Cr <sup>+</sup> , <sup>1</sup> H <sup>37</sup> Cl <sup>16</sup> O <sup>+</sup> , <sup>36</sup> Ar <sup>18</sup> O <sup>+</sup> , <sup>40</sup> Ar <sup>14</sup> N <sup>+</sup>
<sup>56</sup> Fe	<sup>40</sup> Ca <sup>16</sup> O <sup>+</sup> , <sup>40</sup> Ar <sup>16</sup> O <sup>+</sup> , <sup>1</sup> H <sup>37</sup> Cl <sup>18</sup> O <sup>+</sup>
<sup>58</sup> Ni	<sup>58</sup> Fe <sup>+</sup> , <sup>40</sup> Ar <sup>18</sup> O <sup>+</sup> , <sup>40</sup> Ca <sup>18</sup> O <sup>+</sup>
<sup>60</sup> Ni	<sup>44</sup> Ca <sup>16</sup> O <sup>+</sup>
<sup>78</sup> Se	<sup>78</sup> Kr <sup>+</sup> , <sup>40</sup> Ar <sup>38</sup> Ar <sup>+</sup> , Dy <sup>2+</sup> , Gd <sup>2+</sup>
<sup>80</sup> Se	<sup>80</sup> Kr <sup>+</sup> , <sup>40</sup> Ar <sub>2</sub> <sup>+</sup> , <sup>79</sup> Br <sup>1</sup> H <sup>+</sup> , Dy <sup>2+</sup> , Gd <sup>2+</sup> , Dy <sup>2+</sup>
<sup>64</sup> Zn	<sup>36</sup> Ar <sup>14</sup> N <sub>2</sub> <sup>+</sup> , <sup>48</sup> Ti <sup>16</sup> O <sup>+</sup> , <sup>48</sup> Ca <sup>16</sup> O <sup>+</sup> , <sup>32</sup> S <sub>2</sub> <sup>+</sup>
<sup>153</sup> Eu	<sup>137</sup> Ba <sup>16</sup> O <sup>+</sup>

**Table 4. Instrumental Parameters and Operating Conditions for the ICP-MS Instrument**

rf power (W)	1100
nebulizer (carrier gas) flow rate (L min <sup>-1</sup> )	0.90
lens voltage (V)	8.5
analogue stage voltage (V)	-1800
pulse stage voltage (V)	1120
discriminator threshold (V)	70
quadrupole rod offset (V)	0
resolution (amu)	0.70
detector	dual
speed of peristaltic pump (rpm)	24
sweeps/reading	20
replicates	3
dwelt time	50 ms
scan mode	peak hopping
DRC parameters	
CH <sub>4</sub> reaction gas flow (mL/min)	0.70 for Cr, Fe Se, and Zn 1.15 for Sc, Ni, and Eu
rejection parameter <i>a</i> (RPa)	0
rejection parameter <i>q</i> (RPq)	0.5 for Eu 0.6 for Sc, Ni, Se, and Zn 0.7 for Cr and Fe

the RPq value were carried out using matrix blank solution prepared with HCl (2%), HNO<sub>3</sub> (1%), CH<sub>3</sub>OH (1%), Ca at 20 mg/L, Si and Ba at 50 μg/L, and a matrix blank solution spiked with Sc, Cr, Fe, Ni, Zn, and Se at 1 μg/L and with Eu at 0.1 μg/L (see Table 4). The best background equivalent concentrations (BEC) were obtained at a flow rate value of 0.7 mL/min for Cr, Fe, Se, and Zn and at 1.15 mL/min for Sc, Ni, and Eu. The RPq value that led to the best S/N ratio was 0.6 for all elements except Eu (0.5), Cr (0.7), and Fe (0.7). The accuracy and precision of the whole analytical procedure for 35 of the elements monitored (Be, Na, Mg, Al, K, Ca, Sc, V, Mn, Fe, Ni, Co, Cu, Zn, As, Se, Rb, Sr, Y, Cd, Cs, Ba, La, Ce, Nd, Sm, Eu, Gd, Dy, Er, Yb, Lu, Pb, Th, and U) were assessed using the certified reference material (NCS ZC85006 Tomato). Half a gram of the certified reference material was subjected to the digestion treatment under the same conditions as the samples. The extracts were diluted and subjected to ICP-MS analysis.

Accuracy values were considered to be acceptable for 20 elements (values in the range of 70–120%). Analytical figures

of merit for these elements are shown in Table 5. Five elements (Cr, Ga, Ho, Pr, and Tl) with concentration values above the limit of detection (LOD) were added to the 20 elements considered so far. In the assay of <sup>54</sup>Fe, <sup>52</sup>Cr, and <sup>58</sup>Ni polyatomic isobaric interferences had a considerable impact on their signal. Thus, among the 25 elements monitored, 22 elements were finally detected in standard mode (<sup>27</sup>Al, <sup>138</sup>Ba, <sup>44</sup>Ca, <sup>114</sup>Cd, <sup>140</sup>Ce, <sup>164</sup>Dy, <sup>153</sup>Eu, <sup>69</sup>Ga, <sup>158</sup>Gd, <sup>165</sup>Ho, <sup>139</sup>La, <sup>24</sup>Mg, <sup>55</sup>Mn, <sup>23</sup>Na, <sup>142</sup>Nd, <sup>141</sup>Pr, <sup>85</sup>Rb, <sup>152</sup>Sm, <sup>88</sup>Sr, <sup>205</sup>Tl, <sup>89</sup>Y, and <sup>66</sup>Zn) and 3 elements in DRC mode (<sup>54</sup>Fe, <sup>52</sup>Cr, and <sup>58</sup>Ni). The mean concentration values of onion samples for each harvest zone listed in Table 1 and the relative standard deviation can be seen in the Supporting Information.

**Statistical Analysis.** Pattern recognition analysis was carried out using three supervised chemometric techniques (LDA, SIMCA, and BP-ANN) to obtain classification rules for distinguishing between Tropea samples grown according to the production regulations and non-Tropea samples. LDA and SIMCA were applied to the data matrix constituted of the concentration values (μg/kg) of the selected 25 elements expressed on a dry basis. The reliability of the classification rules was validated through a cross-validation procedure. The samples set was randomly divided into a training set and a validation set, the latter containing 1/*k* of the samples (*k* is called the cancellation group). Such a division allows for a sufficient number of samples in the training set and a representative number of members in the validation set. The process was reiterated *k* times with different random constitutions of both sets to ensure that all of the samples were included in the validation set at least once. The goodness of the classification was estimated in terms of prediction ability, which is equal to the percentage of the validation set members correctly classified.

**Linear Discriminant Analysis.** LDA is a chemometric technique that defines a set of delimiters (depending on the number of considered classes) so that the multivariate space of the objects is divided in as many subspaces as the number of classes. Discriminant functions are obtained as a linear combination of descriptor that maximizes the ratio of between-class variance and minimizes the ratio of within-class variance.

LDA is a “hard” classification technique. This means that even if the samples that will be predicted by LDA did not belong to any of the classes of the model, each object will nevertheless be assigned to one of them. Moreover, each object can fall into one and only one class because the multidimensional space is divided into as many subspaces as classes.

Standard and stepwise LDA methods were applied to classify onions according to the categories Tropea (samples grown in accordance with the production regulations) and non-Tropea (samples cultivated in zones different from those cited in the production regulations) used as input a priori. In standard LDA all independent variables were employed in model construction to maintain all of the original information, whereas, in stepwise LDA, the most significant variables involved in sample differentiation were selected using a Wilks’ λ as a selection criterion. An *F* statistic was used to determine the significance of the changes in Wilks’ λ when the influence of each new variable was evaluated. In both cases a 5-fold cross-validation was performed to verify the goodness of the method in terms of classification and prediction ability.

The model obtained by applying standard LDA shows good results for both categories with a mean prediction ability of 94% (Table 6). The forward stepwise analysis (*F* to enter =2.00 and *F*

**Table 5. Summary of Calibration Parameters, Limits of Detection (LODs), Limits of Quantitation (LOQs), and Mean Accuracies Referred to Certified Reference Material NCS ZC85006 Tomato**

isotope	calibration range ( $\mu\text{g/L}$ )	$R^2$	LOD <sup>a</sup> ( $\mu\text{g/kg}$ )	LOQ <sup>a</sup> ( $\mu\text{g/kg}$ )	certified value <sup>b</sup> (mg/kg)	found value <sup>c</sup> (mg/kg)	accuracy (%)	
Al	27	0.1–2000	0.9999	120	144	0.295 (%) $\pm$ 0.043	0.318 $\pm$ 0.005	107.8
Ba	138	0.1–2000	0.9999	1.6	2.5	55.2 $\pm$ 5.2	54.4 $\pm$ 0.7	98.6
Ca	44	0.1–2000	0.9993	4843	7824	5.31 (%) $\pm$ 0.19	4.90 $\pm$ 0.08	92.4
Cd	114	0.1–2000	0.9999	0.75	1.72	0.82 $\pm$ 0.09	0.657 $\pm$ 0.005	80.1
Ce	140	0.01–150	0.9999	0.21	0.24	3.08 $\pm$ 0.22	2.45 $\pm$ 0.26	79.7
Dy	164	0.01–150	0.9999	0.062	0.16	(0.23)	0.19 $\pm$ 0.02	82.6
Eu	153	0.01–150	0.9999	0.0083	0.011	0.048 $\pm$ 0.005	0.05 $\pm$ 0.01	107.1
Fe <sup>d</sup>	54	0.1–2000	0.9999	3898	5365	0.138 (%) $\pm$ 0.015	0.119 $\pm$ 0.006	86.2
Gd	158	0.01–150	0.9999	0.026	0.063	(0.17)	0.179 $\pm$ 0.003	105.3
La	139	0.01–150	0.9999	0.13	0.17	1.78 $\pm$ 0.17	1.31 $\pm$ 0.09	73.6
Mg	24	0.1–2000	0.9999	118	139	0.736 (%) $\pm$ 0.057	0.591 $\pm$ 0.007	80.3
Mn	55	0.1–2000	0.9999	6.78	10.85	87.1 $\pm$ 5.6	68.5 $\pm$ 1.2	78.6
Na	23	0.1–2000	0.9999	88.76	103.94	(0.13)	0.133 $\pm$ 0.003	102.9
Nd	142	0.01–150	0.9999	0.104	0.202	(1.28)	1.11 $\pm$ 0.10	86.4
Ni	58	0.1–2000	0.9999	8.37	9.50	(2.23)	2.51 $\pm$ 0.08	112.5
Rb	85	0.1–2000	0.9999	2.55	5.00	6.66 $\pm$ 0.47	5.39 $\pm$ 0.01	80.9
Sm	152	0.01–150	0.9999	0.020	0.037	0.270 $\pm$ 0.020	0.217 $\pm$ 0.029	80.4
Sr	88	0.1–2000	0.9999	8.59	9.88	569 $\pm$ 40	597 $\pm$ 5	104.9
Y	89	0.01–150	0.9999	0.0722	0.12	(0.675)	0.568 $\pm$ 0.003	84.1
Zn	64	0.1–2000	0.9999	82.88	193.96	36.2 $\pm$ 3.1	27.3 $\pm$ 0.25	75.4

<sup>a</sup> LOD and LOQ values are referred to analysis of tomato samples. <sup>b</sup> Certified values without standard deviation are reported in parentheses. <sup>c</sup>  $\pm$ SD,  $n = 3$ . <sup>d</sup> Analyzed in DRC mode.

**Table 6. Number and Percentage of Correctly Classified Samples (Prediction Matrices) for LDA, Stepwise LDA, and SIMCA Techniques (Rows Represent the True Class, Columns Report the Assigned Class)**

	Standard LDA		Stepwise LDA		SIMCA			
	Tropea	non-Tropea	Tropea	non-Tropea	Tropea	non-Tropea	SENS(%)	SPEC(%)
Tropea	113 (94%)	7	112 (93%)	8	119 (99%)	1	87.5	76.3
non-Tropea	5	75 (94%)	3	77 (93%)	8	72 (90%)	88.8	91.7

to remove = 1.00) has retained 20 variables shown in Table 7. The elimination of redundant information and the selection of only those variables that actually contributed to the discrimination between classes allowed us to obtain only a slight increase of the prediction ability (94.5%, Table 6). These results suggest that the presented method may be successfully used for checking the origin of Tropea red onions. The analyses of both LDA discriminating models show that lanthanides, alkaline metals, and alkaline earth metals are the most important variables in the distinction of geographical origin (Table 7). The importance of lanthanides and in

**Table 7. Summing the Forward Stepwise LDA**

	Wilks' $\lambda$	Parziale Wilks' $\lambda$	F-remove	p level
Dy	0.305893	0.802088	44.16742	0.000000
Sr	0.299931	0.818032	39.81796	0.000000
Ca	0.297713	0.824125	38.19992	0.000000
Rb	0.256867	0.955174	8.40044	0.004220
Ga	0.256274	0.957384	7.96776	0.005301
Tl	0.255014	0.962113	7.04878	0.008647
Ni	0.253719	0.967026	6.10355	0.014427
Ba	0.253205	0.968988	5.72874	0.017723
Nd	0.252872	0.970263	5.48599	0.020269
Zn	0.252487	0.971746	5.20456	0.023705
Ho	0.252418	0.972011	5.15422	0.024382
Al	0.250527	0.979347	3.77495	0.053594
Ce	0.250100	0.981018	3.46351	0.064377
Mn	0.249350	0.983969	2.91635	0.089420
Pr	0.249234	0.984428	2.83144	0.094179
Cd	0.249020	0.985276	2.67503	0.103691
Y	0.248719	0.986467	2.45567	0.118867
Na	0.247657	0.990698	1.68068	0.196503
Mg	0.247585	0.990983	1.62874	0.203530
Cr	0.247027	0.993224	1.22112	0.270625

particular of dysprosium shows that rare earth metals, not considered in the previous study concerning onion authentication,<sup>21</sup> should be taken into account. The important role of alkaline metals and alkaline earth metals in differentiation of cultivation zones is in agreement with our previous studies about the identification of the origin of virgin olive oil, tomato, and tomato paste.<sup>17,18</sup>

It is important to highlight that calcium and strontium have great importance and comparable *F*-remove values. This result is in accordance with literature data, which established that some elements in same group of the periodic table have high concentration correlation in onions due to the similar behavior.<sup>22</sup>

**Soft Independent Modeling of Class Analogy.** SIMCA is a class modeling technique that builds a class model based on the significant principal components (PCs) of category. The range of the scores of the *N* principal components used to build the model for each category are the edges of a hypervolume, the “normal” SIMCA model. Then, in this technique the models obtained for the considered categories can overlap and/or leave some regions of the multivariate space unassigned. Unlike LDA, SIMCA is a soft modeling technique, so an object can belong to one category, to more than one category simultaneously, or to none of the categories. An important consequence of this feature is that SIMCA is able to detect the number of false positives/negatives for each class. Therefore, two parameters can be defined to validate classification: sensitivity (SENS) and specificity (SPEC).<sup>36,37</sup> SENS of a class is referred to the percentage of objects belonging to the class correctly accepted by the class model. SPEC of a class corresponds to the percentage of objects not belonging to the class correctly rejected by the class model.

SIMCA was applied to the same data matrix used in standard LDA, and models obtained were based on five PCs for each class that explain 79.4 and 85% of variance for the category Tropea and non-Tropea, respectively. Validation of the model was carried out by a 5-fold cross-validation procedure. The SIMCA model has provided very good results in terms of mean prediction ability (95.5%) with 99.2% correct prediction for the class Tropea (Table 6). Moreover, the SIMCA model is satisfactory in terms of mean sensitivity (88.5%) and mean specificity (85.5%).

**Artificial Neural Networks.** ANNs are very sophisticated chemometric techniques capable of modeling extremely complex nonlinear functions for classification and regression purposes. Although ANNs are suitable for dealing with issues related to food control and authentication, the diffusion of neural networks for these aims is still at a relatively earlier stage of development.<sup>38</sup> Compared to other multivariate techniques, ANNs operate using a large number of parallel connected simple arithmetic units called neurons. Each neuron is a nonlinear parametrized bounded function, and the pattern of interconnection among them constitutes the network architecture.<sup>38</sup>

Construction of an ANN occurs through the training process using a portion of the data set and by means of opportunely designed training algorithms.

Multilayer perceptrons (MLP) constitute one of the most popular ANN architectures, in which the neurons operating on the same input variable are organized in layers. In particular, feed-forward is a widely used multilayer network architecture in which the information flows only in a forward direction, from the input nodes, through the hidden nodes (if any), and to the output nodes. The numbers of neurons and hidden layers are parameters that determine the predictive efficiency and reliability of network. Each neuron is connected to the neurons of the next layer with different connections called synaptic weights. Learning of the networks is realized by changing the values of all synaptic weights that produce the best fit of the predicted outputs over the entire training data set, and hidden layers are particularly important in coping with nonlinear classification problems.<sup>39</sup>

Before training of a neural network, the proper choice of variables to be used as input is necessary because, if the number of weights is larger than that of samples, “overfitting” may be caused. The inclusion in the data set of redundant, irrelevant, and nondiscriminating variables leads to an increase of noise and may prevent the ANN from finding optimized models. PCA is a widely used multivariate technique for its ability to reduce the dimensionality of a data set retaining most of the information present in the original data. PCA transforms the original variables, using an orthogonal linear transformation, to a new set of uncorrelated variables known as principal components (PCs). Accordingly, PCA has been applied to the concentration of the 25 elements to obtain a dimensionality reduction. The scores of different numbers of PCs were considered as input in the ANN training. The whole data set was split in training and test sets constituted by 160 and 38 samples (cases), respectively. These sets were used for training the network, whereas an independent set of 2 samples (validation set) was used for testing the network prediction ability.

Although the use of a test set allows us to generate unbiased performance estimates, this valuation may exhibit high variance. Then, it is useful to repeat the training procedure a number of times, each time using new training, test, and validation cases to improve reliability and general performance. Overall network performance can be estimated by the mean value calculated from the individual performances. For this, a 100-fold cross-validation was carried out. The obtained performances of networks were carefully checked in terms of magnitude and closeness between the test and validation errors. In fact, if these errors are close together, the network will be able to generalize to new cases; that is, the network will minimize the error on the real error surface. Several tests were conducted to optimize the network in terms of number of nodes in the hidden layer, number of epoch, learning rate ( $\eta$ ), and momentum ( $\alpha$ ) values. Moreover, the selection of the number of PCs as input was also performed to improve the accuracy rate of classification analysis with different BP-ANNs. The order of cases was shuffled at each epoch to ensure more robustness to the back-propagation algorithm and make it less prone to stick in local minima.

The best results were achieved with an MLP 10-7-1 with the first 10 PCs as input variables (explaining 92.8% of total variance) and 100 epochs of back-propagating algorithms. The optimum learning rate and momentum value were  $\eta = 0.15$  and  $\alpha = 0.3$ , respectively. The mean validation performance was 91.5%, and the root-mean-square errors were 0.312 for the training set, 0.484 for the test set, and 0.363 for the validation set.

In conclusion, this work demonstrates that the geographic authentication of the Tropea red onion, recently awarded with PGI certification by the European Union, can be achieved by a simple methodology based on ICP-MS analysis and appropriate statistical treatment of multielement distribution. The results presented above show that discrimination between Tropea red onions and onions from a field not belonging to the cultivation areas specified in the production regulations can be afforded by using different chemometric treatments of data. All four pattern recognition procedures applied (LDA, S-LDA, SIMCA, and ANN) allowed us to obtain satisfactory results because all of the models obtained showed prediction ability >90%. Moreover, in addition to literature data concerning authentication of Japanese onions,<sup>21</sup> this study has demonstrated the importance of the contribution of lanthanides, in particular dysprosium, in the classification of onion origin.



## ASSOCIATED CONTENT

**S Supporting Information.** Mean concentration and relative standard deviation of elements in onion samples. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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