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VALIDATED PUNGENCY ASSESSMENT OF THREE ITALIAN ONION (ALLIUM CEPA L.) CULTIVARS

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Abstract

In the frame of a broad multidisciplinary study aimed at enlightening the peculiarities of three onion cultivars (Dorata di Parma, Borettana and Rossa di Toscana) when harvested in Cannara (Italy), the estimation of pungency (in terms of pyruvic acid content), total titratable acidity, pH, and ascorbic acid content was carried out.-Rossa di Toscana contains the lowest pyruvic acid amount (4.24 mg/100 g fresh weight, FW), whereas no statistically significant differences emernge between the other two varieties (5.41 mg/100 g FW for Dorata di Parma and 4.99 mg/100 g FW for Borettana). The Dorata di Parma shows the highest mean pH value. Accordingly, Dorata di Parma presents the lowest mean acidity with regard to the Borettana and Rossa di Toscana cultivars. The mean ascorbic acid concentration spread between 3.92 and 4.77 mg/100 g FW with no statistically significant differences among the three cultivars.

Key words: *food analysis, italian onion varieties, pungency, pyruvic acid content, varietal differences, method validation*

1. INTRODUCTION

Botanically included in the *Liliaceae* family, onion (*Allium cepa* L.) has been known for thousands of years. Probably originated in central Asia and then introduced in Europe by the Phoenicians around 2000 years ago, onion is grown in every part of the world because it is widely adaptable and can occupy a wide range of ecological niches. World onion production has increased by at least 25% over the past 10 years with current world production being about 85 million tons from 4.3 million hectares making it the second most important horticultural crop after tomatoes (FAOSTAT 2014). Onion is considered one of the most versatile vegetables: from raw to caramelized and from marinated to roasted, it can be found in a wide range of recipes from breakfast to supper and is accepted by almost all traditions and cultures. However, onion is not only a food, but it has also well-known medicinal and functional properties. The recent literature is rich in both *in vivo* and *in vitro* studies reporting the anti-thrombotic- (Lee et al. 2013), hypolipidemic (Lee et al. 2012; Srinivasan 2013), anti-diabetic- (Jung et al. 2011), anti-obesity- (Yoshinari et al. 2012), antioxidant- (Alpsoy et al. 2012; Lee et al. 2012), anti-inflammatory- (Vazquez-Prieto et al. 2013), cancer chemopreventive- (Wang et al. 2012), and antiparasitic properties of onion extracts (Klimpel et al. 2011; Aboelhadid et al. 2013). Moreover, some epidemiological studies suggest that a diet rich in onions may have a favourable effect on the risk of acute myocardial infarction (Galeone et al. 2009) and benign prostatic hyperplasia (Galeone et al. 2007).

Onion's health benefits have been mainly ascribed to two chemical classes of compounds, namely flavonoids and alk(en)yl cysteine sulfoxides (ACSOs) (Griffiths et al. 2002). The first class includes anthocyanins, which impart a red/purple colour to some varieties (such as the typical Italian variety, *Tropea Red Onion*) and flavonols such as quercetin and its derivatives responsible for the yellow flesh and brown skins of many other varieties. ACSOs are odorless, nonproteinogenic sulfur amino acids which are peculiar to the vegetative tissues of all *Allium* species and are indirectly responsible for their characteristic odor and flavor (Rose et al. 2005). Virtanen (1959) first identified (+)-*S*-methyl-*L*-cysteine sulfoxide (methiin), (+)-*S*-propyl-*L*-cysteine sulfoxide (propiin) and (+)-*S*-*trans*-1-propenyl-*L*-cysteine sulfoxide (isoalliin) as the main ACSOs present in onions, with the latter being the most abundant and also the precursor of the lachrymatory factor (LF) (Virtanem et al. 1959). Although stable in intact tissues, ACSOs undergo a rapid enzymatic hydrolysis by the enzyme alliinase (EC 4.4.1.4) as soon as the cells are damaged by mechanical chopping or maceration; indeed, alliinase and ACSOs are present in whole tissues in different compartments, namely vacuoles and cytoplasm, respectively. The initial step in alliinase-mediated ACSOs transformation affords the corresponding sulfenic acids (**1**) and α-iminopropionic acid (**2**) that, spontaneously hydrolyses to give ammonia and pyruvic acid (PA), whereas **1** condenses to form thiosulfinates (TS), in turn generating thiosulphonates, mono-, di- and tri-sulphides as well as the LF (Scheme 1) (Block 1992). The consumer perception of onion pungency depends on these organosulfur metabolites, but since

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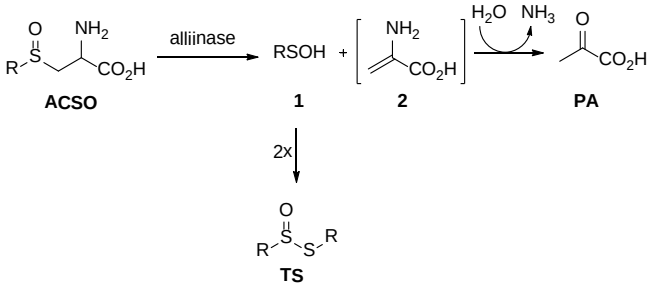
PA is also a final product of this metabolic cascade, its amount is commonly used as a measure of onion pungency.



Scheme 1. Formation of enzymatically produced pyruvic acid (PA).

A vast number of onion varieties are known exhibiting a great diversity in terms of color, shape, dry matter and pungency (Griffiths et al. 2002). Furthermore, the climatic conditions, the agronomic practices and soil type may affect the genetic information of the onion cultivar thus producing a variety of locally present ecotypes endowed with particular organoleptic and nutritional characteristics, often scarcely known outside of the production area. This is the case of Italy where several onion varieties are farmed, although only *Tropea Red Onion*, that was recently awarded with the protected geographical indication (PGI) certification from the European Union, has been the subject of scientific papers (Bonaccorsi et al. 2005; Corea et al. 2005; Dini et al. 2005, 2008a, 2008b; Furia et al. 2011; Gennaro et al. 2002).

Recently, onion cultivars with low pungency (the so-called "sweet" onions) have been increased in popularity among the Italian consumers because they are more attractive for fresh uncooked use. To address these new market demands, Italian onion growers and traders advertise often their products as low-pungency onions without, however, the support of any experimental data. So it is for the three Italian onion cultivars farmed in Cannara, a small town in the Umbria region, well-known since the ancient time for the onion growing, that has developed thanks to the water abundance and the favourable geo-pedological characteristics of the soil. Traditionally, the three varieties produced in the Cannara's area are *Rossa di Toscana*, round and globular with purple tunics, *Borettana*, with a flat, pale yellow bulb and *Dorata di Parma*, characterized by a golden bulb similar to a spinning.

With the aim to initiate a process of characterization of these onion varieties, widely farmed also in other Italian regions, we report herein the assessment of their pungency by the validated quantification of the PA content, along with the determination of total titratable acidity, pH, and ascorbic acid content.

2. MATERIALS AND METHODS

2.1. Onion characteristics

The samples (27) of onion were provided by Cannara Onion Producers' Union (*Consorzio dei Produttori della Cipolla di Cannara*). All the samples were cultivated in 2012 at a farm belonging to Cannara Onion Producers' Union in the municipality of Cannara using the same environmental and agronomic conditions and harvested in July 2012 when 50% of the foliage had collapsed. Afterwards they were stored at an average ambient temperature of 20-25 °C until analyzed (between October 2012 and January 2013). The description of the onion samples, including cultivar, fresh weight, and number of samples, are shown in Table 4. In all the analyses, the normally edible parts of the onions were used, that is the bulb after discard of the outer dry skins, neck and base. All the analyses were performed in triplicate and the results were expressed as mean ± standard deviation.

2.2. Materials

All the reagents were of analytical grade. Sodium hydroxide, metaphosphoric acid, 2,6-dichlorophenol-indophenol (DCIP), 2,4-dinitrophenylhydrazine (DNPH), sodium pyruvate were purchased from Sigma-Aldrich (Milano, Italy). Sodium hydrogen carbonate was purchased from J. T. Baker (Milano, Italy). Hydrochloric acid solution was purchased from Carlo Erba (Milano, Italy). HPLC-grade water was obtained by a New Human Power I Scholar water purification system (Human Corporation, Seoul, Korea).

2.3. Methods

2.3.1. pH and total titratable acidity (TTA)

Half an onion was weighted and homogenized in a domestic electric blender for 2 min without any additional water. Two subsamples were taken from this purée to measure pH and acidity. The pH was determined by potentiometric measurement at room temperature with a pHmeter (Hanna Instruments pH211). The TTA was determined by titration with standardized 0.1 M NaOH by an automated titration system (Mettler Toledo DL22-Food & Beverage Analyzer) equipped with a combined glass pH electrode (Mettler Toledo DG111-SC). The data were expressed as grams of anhydrous citric acid/100 g fresh weight (FW).

2.3.2. Ascorbic acid analysis

Half an onion was weighted and homogenized in a domestic electric blender for 2 min in the presence of 3% aqueous metaphosphoric acid (1 mL/g of fresh onion). The puree thus obtained, was filtered through paper filter by the aid of vacuum. The liquid was transferred into a graduated flask and the volume made up to 100 mL with water. The titration with 2,6-dichlorophenol-indophenol (DCIP) was performed in triplicate on 25 mL of this solution by an automated titration system (Mettler Toledo DL22-Food & Beverage Analyzer) equipped with a double pin platinum electrode (Mettler Toledo DM143-SC). The DCIP solution was prepared by dissolving 0.250 g of DCIP in 500 mL of HPLC-grade water. Sodium hydrogen carbonate (0.21 g) was then added and the resulting solution diluted to 1.0 L with deionized water. The DCIP solution was standardised immediately before use against an accurately weighted sample of ascorbic acid in the presence of diluted sulphuric acid. The data were expressed as mg of ascorbic acid/100 g FW.

2.3.3. Spectrophotometric pyruvic acid analysis

Half an onion was weighted and homogenized without any additional water in a domestic electric blender for 2 min. After 30 min at room temperature the purée was filtered through paper filter by the aid of vacuum. The total volume of the juice thus obtained was measured. The spectrophotometric determination (Anthon, Barrett 2003) of the PA content was performed immediately as follows: a 25 µL aliquot of the onion juice was placed into a glass test tube and 1.0 mL of a solution of 2,4-dinitrophenylhydrazine (DNPH) in 1 M HCl (2.5 g/L) was added. The test tube was then placed into a water bath kept at 37 °C. After 10 minutes, the sample was removed from the water bath and 1 mL of 1.5 M NaOH was then added. The absorbance at 515 nm was then determined within 10 minutes. The standard solutions used in the construction of the calibration curve were prepared following the same procedure by adding 25 µL of sodium pyruvate solution (2, 4, 6, 8 and 10 mM) in place of the onion juice aliquot. The data were expressed as µmol of PA/g FW.

2.4. Method validation

To the best of our knowledge, the conventionally employed spectrophotometric method for the quantitative estimation of PA in onions, has not yet been validated. Thus, in order to assess its statistical value, a full validation study was carried out before the analysis on the investigated onion samples.

The amount of PA in the three onion cultivars was established by using a calibration curve built-up with a series of sodium pyruvate standards previously treated as the real sample, and analyzed in triplicate (Table 1). Specifically, the calibration curve (Eq. 1, Table 1) was constructed by plotting the concentration value of each standard solution (as independent variable) against the corresponding absorbance recorded at 515 nm (in arbitrary units). The obtained mathematical model was characterized by a very appreciable linearity, as indicated by the correlation coefficient value (R^2 = 0.996) and the cross-validation coefficient value (R^2_{xv} = 0.994). The established method was then validated using an external set of three control solutions (3, 5 and 9 mM) whose concentration was included in the above concentration range (Tables 2 and 3).

Cultivar	Bulb weight (g)[a]
Dorata di Parma	122.5 ± 50.8 (71.4-232.9)
Rossa di Toscana	78.6 ± 12.3 (59.32-101.9)
Borettana	79.2 ± 30.2 (40.56-126.42)

[a] Expressed as mean of fresh weight/onion ± SD (minimum-maximum)

Table 1: Description of the Onion Samples.

2.4.1. LOD and LOQ

The LOD and LOQ values were derived from the following Eq. 2 and Eq. 3:

$$C_{LOD} = 3.3\frac{\sigma_y}{b} \quad (2)$$

$$C_{LOQ} = 10\frac{\sigma_y}{b} \quad (3)$$

where C_{LOD} and C_{LOQ} refer to the sample concentration corresponding to the LOD and LOQ, respectively, σ_y is the standard error of the regression equation and *b* is the slope of the regression equation (Eq. 1, Table 1). While LOD resulted equal to 0.7 mM, LOQ was estimated to be 2.0 mM.

2.4.2. Intra-day and inter-day precision

The intra-day precision was assessed by analyzing the three control solutions in triplicate (n = 3) within the same working day. This procedure was repeated for three consecutive days. The previously obtained mathematical model (Eq. 1, Table 1) was then used to calculate the concentrations of the control solutions (mean observed concentration, Table 2). The intra-day precision was determined as the relative standard deviation (RSD%) among the concentration values achieved from consecutive analysis. For each control solution, the variation within replicate analysis performed in a time-frame of three consecutive days (n = 9) was employed to determine the inter-day precision (Table 3). As shown in Table 2, a very appreciable variation in terms of RSD% values was maintained during the consecutive three days of analysis: 3.9%, 3.0%, 2.8% for day 1; 8.5%, 0.8%, 1.9% for day 2; 2.3%, 5.9%, 2.7% for day 3. This, in turn, indicates a high reproducibility of the adopted spectrophotometric method in the short-period. A satisfactory precision was also found when the long-term (inter-day) precision was considered, being the RSD% equal to 6.13%, 4.56% and 2.3% for the solution 1, 2 and 3, respectively (Table 3).

2.4.3. Intra-day and inter-day accuracy

The recovery percentage (recovery%) approach (Sardella et al. 2012) was selected to estimate the accuracy of the adopted method. For each determination, the recovery% value was calculated through the following Eq. 4

$$\text{Recovery\%} = \frac{C_{measured}}{C_{theoretical}}100 \quad (4)$$

where $C_{measured}$ (Table 2) represents the sample concentration as calculated through the regression equation in (mean observed concentration), while $C_{theoretical}$ corresponds to the concentration of the employed external test solution (theoretical concentration).

In analogy to the estimation of the short- and long-term precision, the three control solutions of the external set were also used to estimate the intra-day and the inter-day accuracy. Accordingly, the former was determined by considering the three analysis for each control solution obtained in a single day (n = 3), while the latter was calculated in the time-frame of the three consecutive days of analysis (n = 9).

The recovery% value found for each solution was: 100%, 110%, 101% for day 1; 107%, 106%, 101% for day 2; 113%, 112%, 103% for day 3. Good accuracy was also achieved in the long-period, being the recovery% values equal to 107%, 108% and 102% for solution 1, 2 and 3, respectively (Table 3).

Compound	Regression Equation	R^2	R^2_{xv}	Eq. #	Linearity range (mM)
Sodium pyruvate	y = 0.0087 (± 0.0028) + 0.0226 (± 0.0004) x	0.996	0.994	1	02-10

Table 2: Calibration data for sodium pyruvate derivatized: regression equation, correlation coefficient (R^2) value, cross validation coefficient (R^2_{xv}), explored linearity range.

Solution	Theoretical concentration (mM)	Day	Mean observed concentration (mM)	n[a]	Precision (RSD%)	Accuracy (Recovery %)
1	3.0	1	3.0	3	3.9	100
		2	3.2		8.5	107
		3	3.4		2.3	113
2	5.0	1	5.5	3	3.0	110
		2	5.3		0.8	106
		3	5.6		5.9	112
3	9.0	1	9.1	3	2.8	101
		2	9.1		1.9	101
		3	9.3		2.7	103

[a] Number of replicates

Table 3: Statistical analysis for sodium pyruvate derivatized in the short period (intra-day precision and accuracy values).

2.5. Statistical analysis

Three onion bulbs of each cultivar were analyzed in triplicate for each assay. All of the statistical analyses were performed by means of the SigmaPlot software for Windows. The Shapiro-Wilk test was applied to verify whether the distribution of the variables was normal ($p < 0.05$). Mean values obtained for the variables studied in the different groups were compared by one-way analysis of variation using ANOVA and ANOVA on Ranks when the statistical distribution was not normal (Tukey's multiple comparison test). Significant differences among them were assumed when the statistical comparison gave $p < 0.05$.

3. RESULTS AND DISCUSSION

The major aim of this study was the evaluation of the pungency of *Dorata di Parma*, *Rossa di Toscana*, and *Borettana* varieties. From the business point of view, the pungency is a very important feature of an onion variety because generally high pungent cultivars did not appeal to the consumers which have been oriented toward mild onions. A pungency value can be assigned to an onion by the breeder following a taste evaluation by a panel or, indirectly, through the measurement of the PA content, produced by the enzymatic breakdown of ACSOs. Following the procedure described in details in the Experimental, we determined the PA content by the spectrophotometric method.

Solution	Theoretical concentration (mM)	Mean observed concentration (mM)	n [a]	Precision (RSD%)	Accuracy (Recovery %)
1	3.0	3.2		6.13	107
2	5.0	5.4	9	4.56	108
3	9.0	9.2		2.3	102

[a] Number of replicates

Table 4: Statistical analysis for sodium pyruvate derivatized in the long period (inter-day precision and accuracy values).

Cultivar	Pyruvic Acid (mg)[a]
Dorata di Parma	5.41 ± 0.46 (5.08-5.85)
Borettana	4.99 ± 0.43 (4.54-5.27)
Rossa di Toscana	4.24 ± 0.56 (3.66-4.57)

[a] Expressed as the mean PA/100 g FW, ± SD (minimum-maximum)

Table 5: Pyruvic Acid Content in *Dorata di Parma*, *Rossa di Toscana* and *Borettana* Onions.

The mean PA values varied significantly between *Dorata di Parma* and *Rossa di Toscana* and between *Borettana and Rossa di Toscana*, whereas no significant difference exists between *Dorata di Parma* and *Borettana*, which showed a PA content higher than the red tunic cultivar, *Rossa di Toscana*. Recently, the PA

content of a sample set consisted of white, yellow and red onions cultivated in Italy, has been correlated to the bulb tunic colours (Gallina et al. 2012). As in the paper there is no mention of the bulb varieties analyzed, a direct comparison of our results with those data should be misleading. However, the yellow and red onion clones analyzed by Gallina (2012) had a PA content covering a range from 4.9 to 11.7 µmol/g FW (mean = 8.9 µmol/g FW), and from 4.6 to 10.9 µmol/g FW (mean = 8.8 µmol/g FW), respectively. They found the highest distribution in the range of 6-8 µmol/g FW for yellow clones as well as for the red ones. It is interesting to note that not one of the 41 yellow phenotypes analyzed by Gallina (2012) was endowed by a PA content in the range of 4-6 µmol/g that is the interval including our yellow cultivars *Dorata di Parma* and *Borettana*. Also the red cultivar *Rossa di Toscana* takes place among the mildest clones of its corresponding category. The peculiar "mildness" of the varieties we analyzed may derive, at least in part, from the geographical origin of our samples. Indeed, the town of Cannara rises on the bank of a river and the soil where the onions are grown is very rich in water; moreover, it has been reported that among the non–genetic factors affecting pungency in onions, water stress has to be considered (Orzolek 2010). According to the onion industry that classifies onions in low pungent (0-3 µmol/g FW), moderate pungent (3-7 µmol/g FW) and high pungent (>7 µmol/g FW), *Dorata di Parma*, *Borettana* and *Rossa di Toscana* farmed in Cannara can be considered as moderate pungent cultivars.

All the onion samples were slightly acidic: the pH spread over a relatively small range, between 5.42 and 5.88, which agrees with the data reported in the literature for other varieties (Rodríguez Galdón et al. 2008). The *Dorata di Parma* cultivar showed the highest mean pH value, with statistically significant differences with respect to the mean values obtained for the other two cultivars (Table 5). About TTA, according to the data of pH, the *Dorata di Parma* cultivar had the lowest mean acidity with statistically significant differences ($p < 0.05$) with regard to the *Borettana* and *Rossa di Toscana* varieties. These data were in line with those reported in the literature for other cultivars and expressed in the same units as our results, namely grams of citric acid /100 g FW (Rodríguez Galdón et al. 2008).

Cultivar	**pH**[a]	**TTA (g)**[b]	**Ascorbic Acid (mg)**[c]
Dorata di Parma	5.70 ± 0.18 (5.53-5.88)	0.1049 ± 0.0078 (0.0987-0.1137)	3.92 ± 1.92 (2.50-6.10)
Rossa di Toscana	5.48 ± 0.02 (5.46-5.50)	0.1266 ± 0.0139 (0.1130-0.1408)	4.77 ± 1.78 (3.32-6.75)
Borettana	5.47 ± 0.08 (5.42-5.56)	0.1257 ± 0.0095 (0.1149-0.1328)	4.70 ± 0.87 (3.70-5.20)

[a] Expressed as mean ± SD (minimum-maximum). [b] Expressed as mean anhydrous citric acid/100 g FW, ± SD (minimum-maximum). [c] Expressed as mean ascorbic acid/100 g FW, ± SD (minimum-maximum)

Table 6: pH, Total Titratable Acidity (TTA) and Ascorbic Acid Content in *Dorata di Parma*, *Rossa di Toscana* and *Borettana* Onions.

Although the contribution to the daily vitamin C intake from the consumption of an onion serving (100 g) is considered relatively low, representing less than 7% of the recommended dietary intake, we decided to determinate the ascorbic acid content all the same because no data on these Italian onion varieties are reported. The mean ascorbic acid concentration obtained after DCIP titration procedure spread between 3.92 and 4.77 mg/100 g FW with no significant differences among the three analyzed cultivars. The values we found were similar to those obtained by Rodríguez Galdón (2008), Franke (2004), and Proteggente (2002) but higher than the data reported by other investigators (Gökmen et al. 2000; Pelledrini et al. 2007).

4. CONCLUSIONS

The evaluation of three Italian onion varieties, namely *Dorata di Parma*, *Borettana* and *Rossa di Toscana*, in terms of pungency, pH, TTA and ascorbic acid content has been reported. This is the first work correlating such data with a specified Italian onion cultivar. Among the three varieties, the red one, *Rossa di Toscana*, is endowed with the lowest PA content, whereas no statistically significant difference has been observed between the other two varieties, both characterized by golden tunic. However, all the samples analyzed in this study resulted quite low in PA content (4-6 µmol/g FW); therefore *Dorata di Parma*, *Borettana* and *Rossa di Toscana* farmed in Cannara (Italy) can be classified as medium pungent varieties. It may be hypothesized that the geographical origin of our samples, contribute to the mildness of the onions. Moreover, for the first time the spectrophotometric method conventionally applied for the estimation of the pyruvic acid content in onions, was fully validated.

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