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Free amino acid profile of *Malus domestica* Borkh cv. Annurca from the Campania Region and other Italian vegetables

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Abstract

The apple *Malus domestica* Borkh cv Annurca is a variety from the Campania Region (Southern Italy) known for its high quality and recognized with the "Protected Geographical Indication" (PGI) trademark. Differences in cultivation area could help to guarantee the geographic origin of this product. Free amino acids of Annurca apples cultivated in the Campania Region were determined on an amino acid analyzer with post-column ninhydrin derivatization procedure and utilized in a chemometric approach. The main free amino acids present in samples from Annurca apples were asparagine (>70 mg/100 g), aspartic acid, glutamic acid and o-phosphoserine (about 1-5 mg/100 g each). Other amino acids were detected in minor amounts (less than 1 mg/100 g). Subsequently, the cluster analysis indicated that the Annurca apples from Presenzano, Pietravairano, Vitulazio and Valle di Maddaloni (little villages of the Campania Region) may be distinguished on the basis of their free amino acid profiles. The confidence of the cluster obtained for apples, was tested considering the free amino acid profiles of other Italian vegetables. It is concluded that free amino acid profiles are good indicators of the geographic origin of Annurca apples and other vegetables, proposing a simple method compared with more tedious ones, which could be transferred to other laboratories, making easier the acquisition of raw data (free amino acid profiles).

Keywords: Apple; cluster analysis; free amino acid; *Malus domestica* cv. Annurca.

Abbreviations: *nor*-Leu, *nor*-leucine; PCA, Principal Component Analysis; PCO, Principal coordinates analysis; PITC, phenylisothiocyanate. For the amino acids the standard three-letter code has been used.

Introduction

Consumers are showing an increasing interest in the geographical or varietal origin of foodstuffs they buy; this information is regarded as an additional warranty of their quality and authenticity. This requirement is expanding to various products, including vegetable and fruit. In fact, in some Italian regions, the promotion of the Denomination of Protected Origin (DOP) and Protected Geographical Indication" (PGI) certifying vegetables and fruits authenticity is requested by producers. It could be important to facilitate DOP and PGI for production of typical vegetables and fruits, linked to the local production and to traditional working techniques. For this purpose, it is necessary to set up objective tests to verify the authenticity of geographical origin. Therefore, analytical monitoring is necessary on vegetable and fruit samples produced in different geographical areas to find authenticity markers that could be employed to characterize typical products. Multivariate techniques, such as DNA (Kumar et al., 2009; Theocharis et al., 2010) or chromatography (Merken and Beecher 2000) or electrophoresis (Faisal Anwar Malik et al., 2009) based methods, have been shown to be an interesting set of powerful tools to analyse and recognise hidden patterns in complex matrices (Martinez et al., 2003) and in this way to characterise similar cultivars (Burstin and Charcosset 1997). Chemometric methodologies applied to food composition, proved that the geographical and varietal origin of the samples can be related to chemical compounds. Amino acids are a component of many natural foods, and, although their percentage is affected by many factors, such as fruit maturity (Sano and Kawashima 1982) mineral nutrition (Devitt et al., 1987; Foyer et al., 2003), water stress (Good and Zaplachinski 1994), climatic conditions, field treatments and light-dark transitions (Fritz et al., 2006), the relationship between their levels remained rather similar (Fritz et al., 2006). Indeed, some researchers have successfully used free amino acid contents to determine geographical origin and varieties of wines (Kyoung Rae et al., 1996; Soufleros et al., 2003), legumes (Baudoin and Maquet, 1999), almonds (Helena Seron et al., 1998), peanuts (Andersen et al., 1998) and apple juice (Pilando and Worlstad. 1992). To the same aim, in the present paper we have performed multivariate analyses on *Malus domestica* cv Annurca, which is the most common apple in the Campania Region, representing about 10% of the national apple production, 90% of which comes from the Campania Region (Floris 1997), especially from the Caserta province. This cultivar has been given the PGI (Protected Geographical Indication) trademark "Melannurca Campana", within the European Council project related to the preservation of local and characteristic agriculture commodities (CEE rule nr. 2081, 1992). Therefore, determining the origin of "Melannurca Campana" apple is an important objective for the local economy. Previous work,

Table 1. Cultivars used in this study

Species	Cultivar	Source	Samples analysed	Used portion
Malus domestica	Golden	Commercial	3	Apples
M. domestica	Annurca	Presenzano (CE)	3	“
M. domestica	Annurca	Vitulazio (CE)	3	“
M. domestica	Annurca	Vairano (CE)	3	“
M. domestica	Annurca	Valle di Maddaloni (CE)	3	“
Asparagus officinalis		Commercial	3	Sprouts
A. acutifolius		Colli Tifatini (CE)	3	“
A. acutifolius		Raviscanina (AV)	3	“
Cynara scolymus	Capuanella	Caserta	3	Heads
Lathyrus sativus		Valle Agricola (CE)	3	Seeds
Solanum tuberosum	®	Caivano (CE)	3	Tuber
S. tuberosum	®	Gallo Matese site 1 (CE)	3	“
S. tuberosum	®	Gallo Matese site 2 (CE)	3	“
S. tuberosum	®	La Spezia	3	“
S. tuberosum	Merit	Commercial	3	“
S. tuberosum	Agria	Commercial	3	“

®: product typical of the site without documented information.

using DNA fingerprinting, described the genetic authentication of fruits from “Annurca” and “Annurca Rossa del Sud”, not considering the origin of the cultivar (Melchiade et al., 2007). Some researchers showed the presence of free amino acids from different apple varieties (Vasanits and Molnar-Perl 1999; Elbert and Esselen 2006; Bruckner and Westhauser 2003). In particular, the separation and identification of free amino acids were obtained by RP-HPLC and PITC pre-column derivatization, respectively (Vasanits and Molnar-Perl 1999; Vasanits et al., 2000). The RP-HPLC allows separation of twenty-seven phenylthiocarbamyl-amino acids, optimizing various parameters such as temperature, flow-rate and eluents.

In this study, we report data on the free amino acid content of *M. domestica* cv Annurca fruits from four different Campania districts (Presenzano, Pietravairano, Vitulazio and Valle di Maddaloni). The free amino acid content was determined, after direct extraction, by cation exchange liquid chromatography and post-column ninhydrin derivatization (Stein and Moore 1963). Subsequently on these raw data we performed a cluster analyses to verify the geographic origin of the cultivar (Jacobsen and Gunderson 1986). The aim of this paper was to study the free amino acid profile in different apples to verify sources of differentiation. Furthermore, to verify the confidence of the obtained cluster, we included the free amino acid profiles determined for other Italian vegetables.

Materials and methods

Chemicals and reagents

Sulfosalicylic acid, ethanol and standard amino acids were from Sigma-Aldrich (Milan, Italy). All chemicals and solvents for the automated amino acid analysis were obtained from Biochrom (Cambridge, UK). The internal standard (*nor*-Leu) was purchased from Mann Research Laboratory (New York City, NY, USA).

Plant material

The edible part of cultivars were studied (the number of cultivar(s) analyzed is reported in parentheses; Table 1): asparagus (3) [*Asparagus officinalis* L. (cultivated asparagus), *Asparagus acutifolius* L. from Colli Tifatini, Caserta (wild asparagus) and *A. acutifolius* from Raviscanina, Caserta], apples (2) [*Malus domestica* cv Golden and Annurca], artichoke (1) [*Cynara scolymus* cv. Capuanella],

Fig 1. Caserta province (Campania, Italy). Numbers indicate the provenance areas of *M. domestica* cv. Annurca sampling: 1, Presenzano; 2, Pietravairano; 3, Vitulazio; 4, Valle di Maddaloni.

grass pea (1) [*Lathyrus sativus*] and potato (6) [*Solanum tuberosum* from Gallo Matese (two different sites) (Caserta), *S. tuberosum* from Caivano (Naples), *S. tuberosum* from Veppo (La Spezia), *S. tuberosum* cv. Agria (commercial), *S. tuberosum* cv. Merit (commercial)]. The analysis were made on asparagus apical edible part (turion), peeled apples without endocarp, artichoke heads, grass pea seeds and peeled potato.

The samples of Annurca apples were collected from four different Caserta (Campania, Southern Italy) orchards: Presenzano, Pietravairano, Vitulazio and Valle di Maddaloni (Fig 1). In particular, samples of *M. domestica* cv Annurca were harvested from six different trees grown under the same conditions in each district. A total of 24 samples were analyzed. Golden apples, were purchased in three different supermarkets, as control samples.

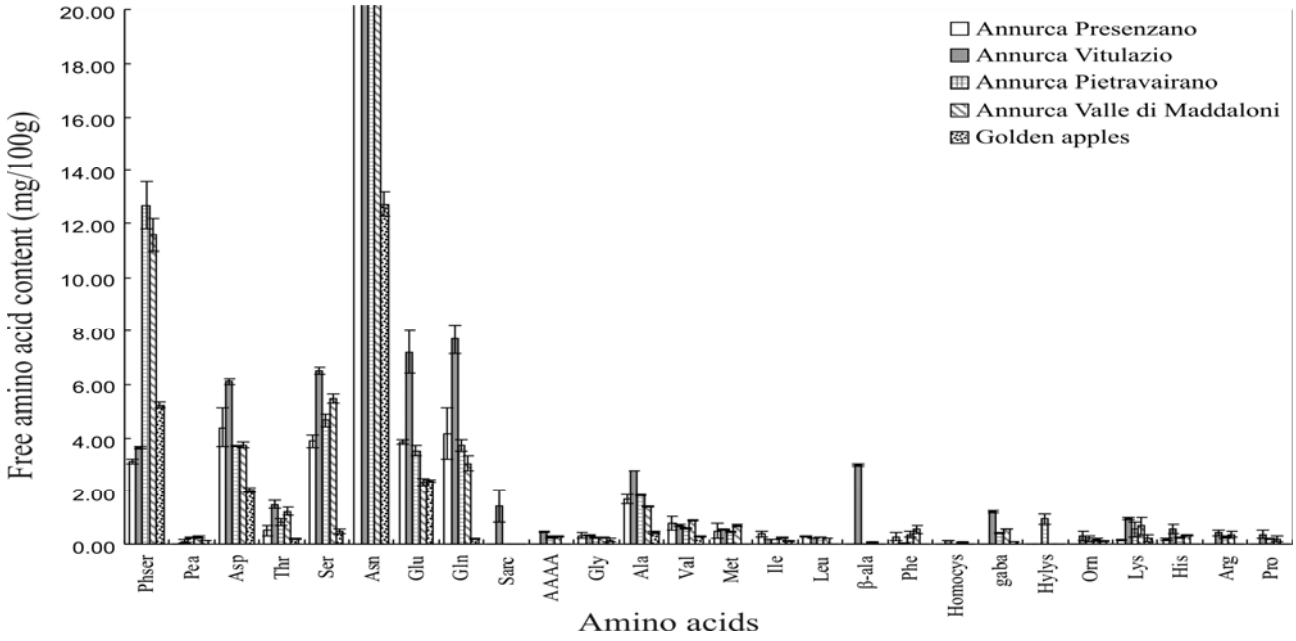

Fig 2. Free amino acid content of extracts from commercial Golden and Annurca apples collected in Presenzano, Vitulazio, Pietravairano, Valle di Maddaloni. Results are expressed as milligrams of amino acids per 100 g of sample. For Asn values see text.

For the analysis, samples were cleaned with MilliQ water, drained, and gently blotted with a paper towel. Each sample was then powdered with porcelain mortar and pestle (i.d. 130 mm, 320 mL) in liquid N_2, until particles of homogeneus size were obtained. Samples were then transferred into 50 mL polypropylene bottles (Falcon, Becton Drive, Franklin Lakes, NJ USA), covered with silver paper and stored at -80°C until use. In particular, on the Annurca apples of Presenzano, Pietravairano, Vitulazio and Valle di Maddaloni collected at the same level of maturity, we quantified the sugar content (°Brix). For the analyses, 10.0 g of each frozen powdered apple were homogenized for 1 min in a Waring Blendor with 10.0 mL of MilliQ water; then centrifuged, using a Beckman GS-15R centrifuge (Beckman Coulter, Milan, Italy), for 5 min at 4200 x *g*. The values of degrees °Brix were measured at RT on the supernatant apple juice using a Mod. 101 Sinergica refractometer within a measuring range of 0-32 °Brix (Sinergica Soluzioni S.r.l. Montesilvano, PE, Italy).

Free amino acid extraction of apples and other vegetables

For the analysis of free amino acids, edible part aliquots of about 200 mg of frozen powdered apple, asparagus, artichoke, grass pea and potato samples were precipitated with 80% cold ethanol (1.0 mL), in the presence of nor-Leu (50 nmol) as internal standard, homogenized with a teflon pestle and centrifuged at about 14000x*g*, at 4 °C. The supernatant was lyophilized, treated with 6% sulfosalicylic acid (500 µL) to precipitate any protein fraction still present, and centrifuged again (Iriti et al., 2005). Generally, 30 µL of this extract were directly analyzed. Each sample was individually prepared and analyzed in triplicate.

Amino acid analyses

A Biochrom 20 (Cambridge, UK) amino acid analyzer, equipped with a polyvinyl sulfonate cationic-exchange column for physiological fluids, a post-column ninhydrin derivatization system and a two-channel detection system set at 570 and 440 nm (the second for proline and hydroxyproline) was used, adapting the Stein and Moore procedure (1963). The amount of each amino acid was expressed as mean percentage. Using this method, we obtained the total free amino acid content with a single analysis, simplifying the acquisition of raw data. For the free amino acids the standard codes were used**:** 1-mhis: 1-methyl-L-histidine; 3-mhis: 3-methyl-L-histidine; AAAA: L-α-aminoadipic acid; Aaba: L-α-amino-n-butyric acid; Ala: L-alanine; Asn: L-asparagine; Arg: L-arginine; Asp: L-aspartic acid; Car: L-carnosine; Citr: L-citrulline; Cys: L-half cystine; Cysth: cystathionine; Ethan: ethanolamine; GABA: γ-amino-n-butyric acid; Gln: L-glutamine; Glu: L-glutamic acid; Gly: glycine; His: L-Histidine; Hylys: δ-hydroxylysine; Hypro: hydroxyl-L-proline; Ile: L-isoleucine; Leu: L-leucine; Lys: L-lysine; Met: L-methionine; Orn: L-ornithine; Pea: o-phosphoethanolamine; Phe: L-phenylalanine; Phser: o-phospho-L-serine; Pro: L-proline; Sarc: L-sarcosine; Ser: L-serine; Taur: taurine; Thr: L-threonine; Trp: L-tryptophan; Tyr: L-tyrosine; Val: L-valine; β-ala: β-alanine.

Statistical analysis.

The samples from the four districts were subjected to statistical analyses, to investigate and point out the underlying data structure. Data were pre-processed (standardized): the absolute concentrations were scaled using the total amino acid concentration.

Data were analyzed with the ANOVA (analysis of variance) test for the comparison of each amino-acid concentration mean. In order to identify groups related to the free amino acid profiles, a multivariate cluster analysis was carried out, using the commercial statistical package PAST (http://folk.uio.no/ohammer/past/).

Dendograms were built using clustering with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The clusters were joined using Principal Coordinate Analysis (PCO), Spearman correlation rho and graphical tools such as biplots. Principal coordinates analysis (PCO) is an ordination method that finds the eigenvalues and eigenvectors of a matrix containing the distances between all data points (Davis, 1986). Correlation using Spearman's rho allows finding hierarchical groupings in multivariate data sets.

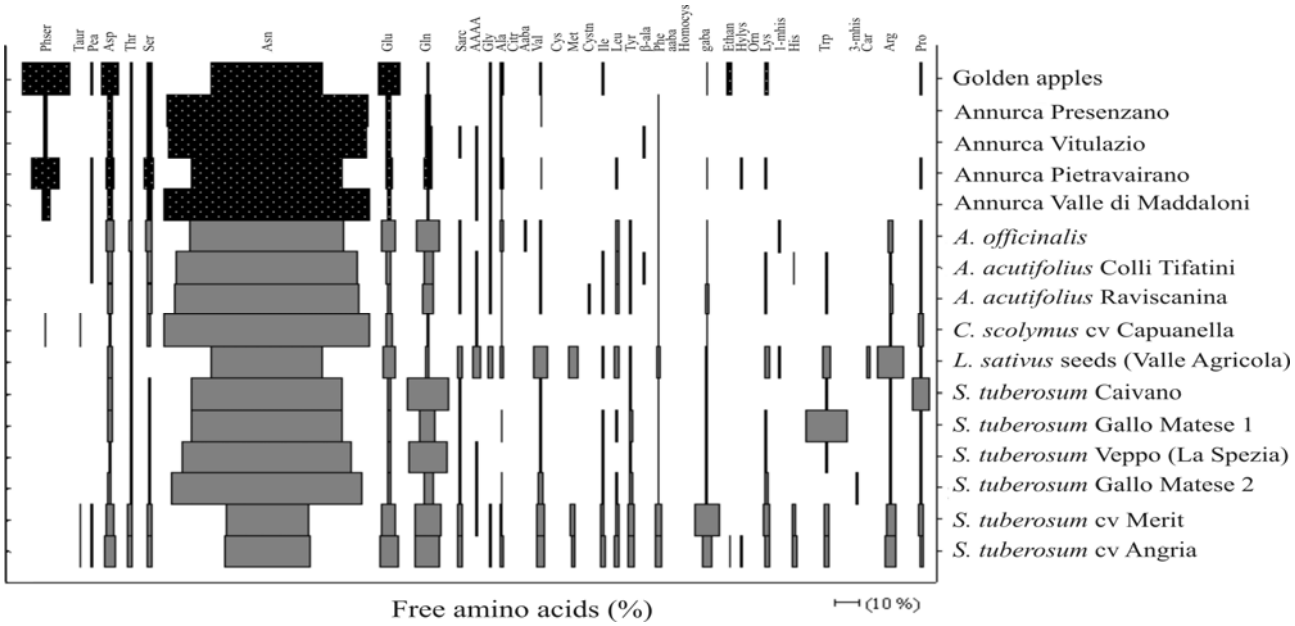

Fig 3. Spindle diagram showing the free amino acid profiles present in the analyzed species. In this diagram, the vertical axis represents the species, while the horizontal axis represents the percentage of single free amino acid. Width of bars is proportional to the percentage of amino acid content.

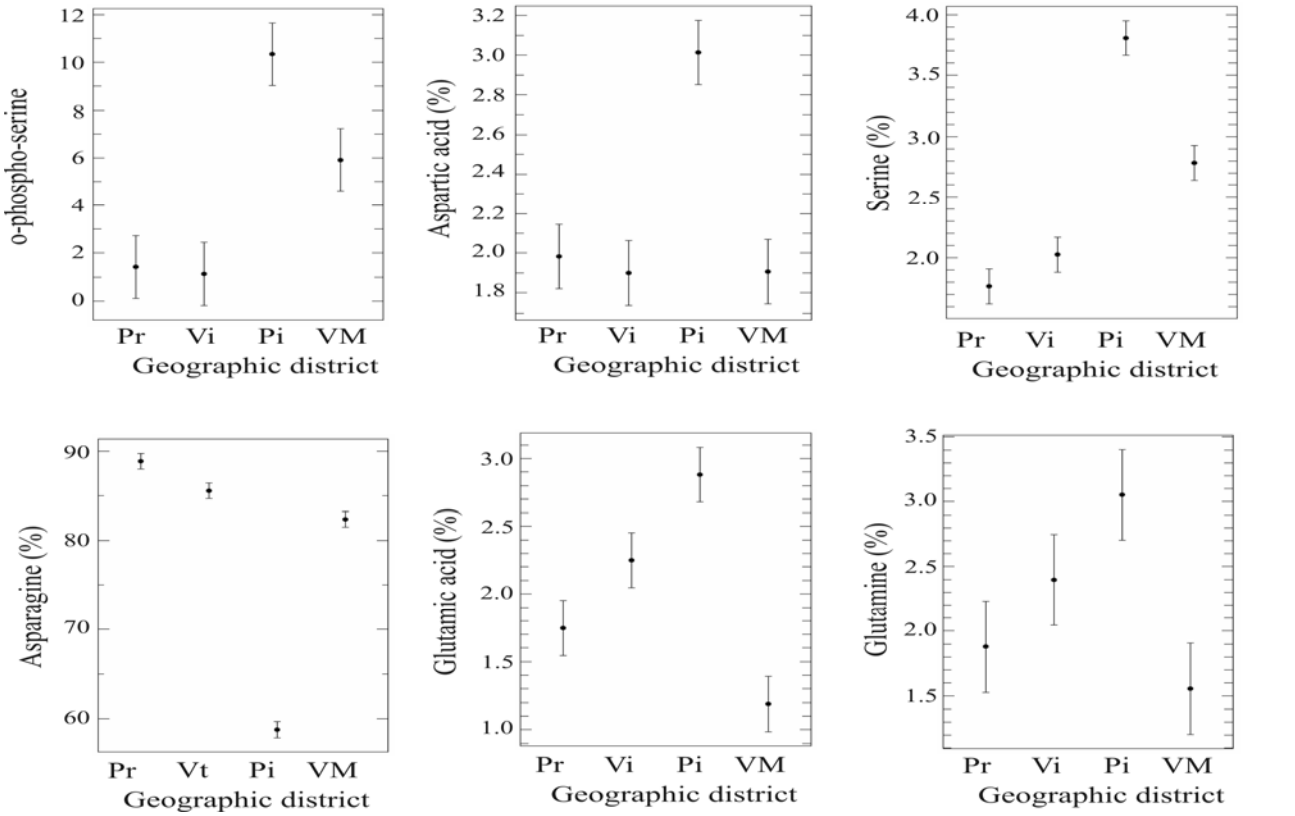

Fig 4. Box plot of six amino acids with percentages higher than 1% in Annurca apples from the four geographical districts. Mean values (●) were significantly different with ANOVA test. Axis Y, relative composition percentage; axis X, sampling district (Pr, Presenzano; Vi, Vitulazio; Pi, Pietravairano; VM, Valle di Maddaloni).

Results

Free amino acids in M. domestica cv Annurca

The free amino acid profile was obtained using the previously described method (Iriti et al., 2009; Vannini et al., 2006) on peeled apples cv. Annurca from four Campania districts: Presenzano, Vitulazio, Pietravairano and Valle di Maddaloni (Fig 1). T he amount of free amino acids, expressed as mg/100g of fresh product, is reported in Fig 2. Both qualitative and quantitative differences were found between Annurca and Golden apples. Total free amino acid mean content in 100 g of fresh Annurca apple was higher in Presenzano, Vitulazio, Pietravairano and Valle di Maddaloni (220.53± 13.08, 320.48± 5.53, 122.08± 5.90, 196.18± 3.67

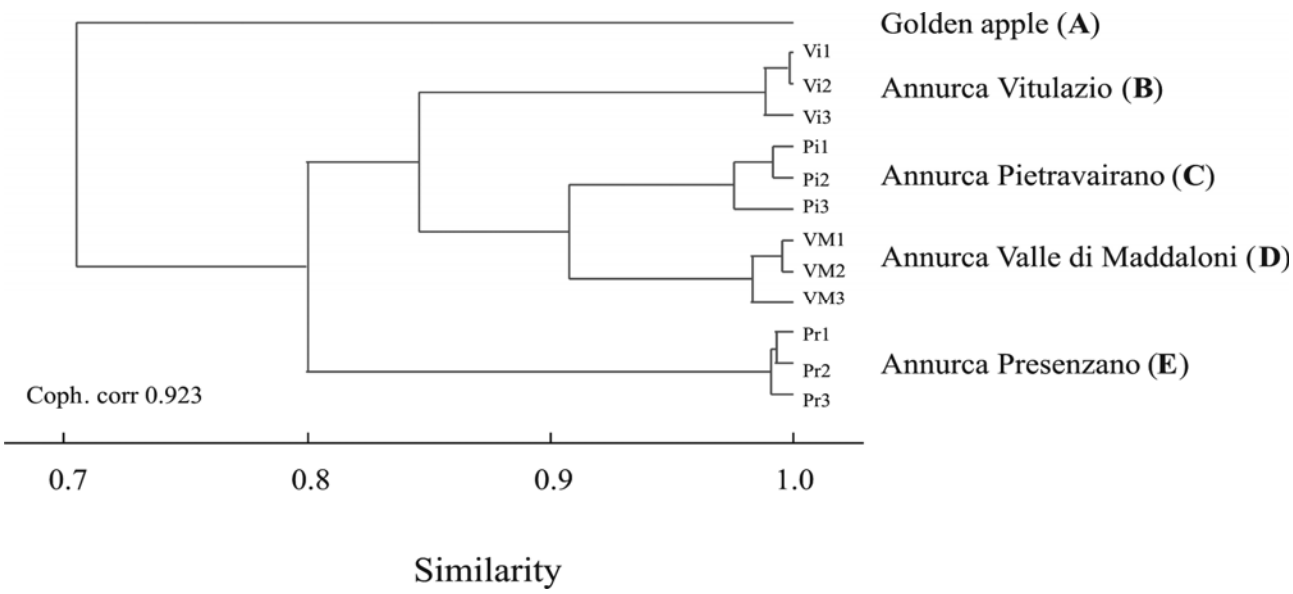

Fig 5. Dendrogram corresponding to the cluster analysis for free amino acid profiles in cv. Annurca apples from four different Caserta district (Campania, Italy) growing fields: Presenzano (Pr), Pietravairano (Pi), Vitulazio (Vi) and Valle di Maddaloni (VM). Scale represents the distance between the groups.

mg/100 g, respectively) than Golden apple (25.33±0.73 mg/100 g).

Asparagine in Annurca apples was by far the most abundant free amino acid [about 196.00 ± 12.17 (89% of total), 274.14±3.52 (86%), 71.71±2.10 (59%) and 161.38±2.77 (82%) mg/100 g in Presenzano, Vitulazio, Vairano and Valle di Maddaloni apples, respectively].

The most abundant free proteic amino acids in Annurca apples (> 1mg for 100 g), were Asp, Ser, Glu, Gln and Ala. The samples contained also non-protein amino acids. In particular, phosphoserine (Phser) was the most abundant in Annurca apple (3.13, 3.62, 12.69 and 11.59 mg/100 g in Presenzano, Vitulazio, Vairano and Valle di Maddaloni apples, respectively). The amount of each of the other non-protein amino acids did not exceed 3 mg/100 g of fresh product. Trp, Tyr, Ans, Hypro, Aaba, Cysth, Cys, Taur, 1-mhis, 3-mhis, Car and Citr were not detected in both apples (Annurca and Golden).

The Spindle diagrams of free amino acids of Annurca apple fruits from the four districts and that of Golden apple fruits, are shown in Fig 3. There is a significant difference in Annurca and Golden apples due to the higher or lower percentages of different amino acids.

Cluster analysis of free amino acid profiles in M. domestica cv Annurca fruits

First, a comparison of the percentage composition of all amino acids in the apples from the four districts (Presenzano, Vitulazio, Pietravairano and Valle di Maddaloni) was performed. In particular, Fig 4 shows the box plot analysis of the most abundant amino acids (aspartic acid, asparagine, glutamic acid, glutamine serine and o-phosphoserine). There was a significant difference in the percentage composition of different amino acids between districts. The relative cluster analysis is shown in Fig 5. It can be seen that the sample point arrangement is not random. Moreover, the dendrogram suggested five groups (A-E) of free amino acid profiles. The amino acid profile of cluster A (Golden apple) differed (Fig 5) significantly from Annurca apple clusters (B-E). Indeed, the three different profiles obtained from each district form a specific cluster. Thus, the data show the likely cultivation area of different Annurca apples, using reference profiles.

A final consideration worthy of mention is that, in this work, only one kind of cultivated apple (Golden) was analyzed. Therefore, the comparison between species here reported should not be considered as conclusive.

Cluster analysis of free amino acid profiles in M. domestica apple and other vegetables

Finally, to confirm the confidence of the cluster obtained for *M. domestica* apples, the same statistical analysis was applied to the free amino acid profiles from other vegetables. The following free amino acid profiles were used: *A. officinalis*, *A. acutifolius*, *C. scolymus* cv Capuanella, *S. tuberosum* (cv Merit and Agria) and other *S. tuberosum* from various Italian districts (Table 1).

In Fig 3, the Spindle diagram showed that the free amino acid profiles of apple, asparagus, artichoke, grass pea and potato samples, contained high amounts of asparagine, glutamic acid and glutamine. All the other amino acids were present in lower quantities. However, differences exist in the relative amounts of each amino acid. To ascertain if it may be possible to differentiate between the reported species, a cluster analysis was performed (Fig 6). As previously reported, samples were not randomly grouped. Moreover, in agreement with grouping criteria, the statistical analysis suggested three main clusters (A, B and C). Cluster C included samples of different apples (Annurca and Golden), cluster B included potato samples of different varieties and districts, and cluster A included asparagus samples. *C. scolymus* cv Capuanella and *L. sativus* seeds (Valle Agricola) were separated from the other considered species. Furthermore, this analysis again confirmed the different profiles of Annurca apples from Presenzano, Pietravairano, Vitulazio and Valle di Maddaloni districts.

These findings were also confirmed by the Principal Coordinate Analysis (PCO) (Fig 7), which clearly segregated the *M. domestica* cv Annurca apple into a well-defined group, together with Golden apple in a larger circle (circle A1 and A in Fig 7, respectively). PCO also confirmed the grouping of the potato and asparagus species. *L. sativus* seeds and *C.*

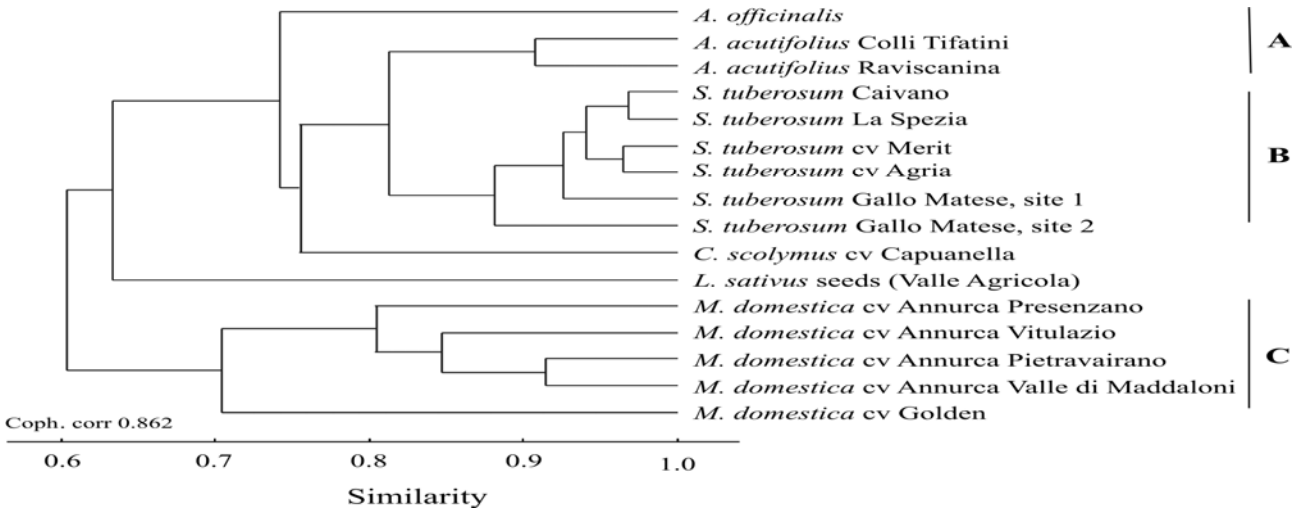

Fig 6. Dendrogram corresponding to the cluster analysis for free amino acid profiles in: *A. officinalis* (commercial), *A. acutifolius* (Colli Tifatini and Raviscanina), *M. domestica* (cv Annurca and Golden), *C. scolymus* cv Capuanella, *L. sativus* seeds (Valle Agricola), *S. tuberosum* from Gallo Matese (sites 1 and 2), Caivano, Veppo (La Spezia), cv. Agria and cv. Merit (commercial). Scale represents the distance between the groups.

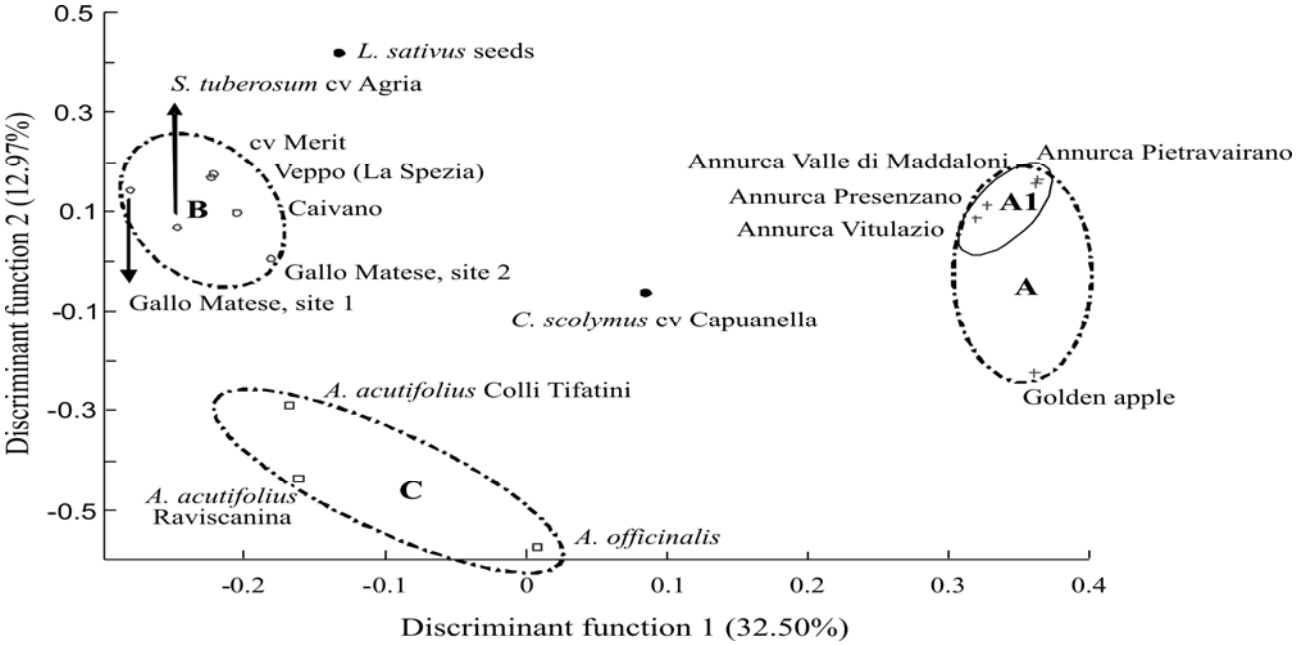

Fig 7. Principal coordinate analysis obtained by free amino acid profiles of the cultivated species reported in Table 1. The analysis showed a segregation of apples and other vegetables into characteristic groups.

scolymus cv Capuanella were well separated.

Discussion

As retailers and consumers have become increasingly demanding about what they sell and buy, precise identification of edible plants and labelling of food products have become important issues in agricultural and food sciences. For this reason, novel protocols have been proposed to authenticate commercial agricultural products. Free amino acids represent a consistent fraction of many natural foods, and sometimes their amount has been exploited for differentiation purposes. In many papers, the ability of free amino acids to establish significant differences within a number of the same cultivars from the same region has been reported (Davies 1976; Helena Seron et al., 1998; Tomàs and Molins 1990; Varela et al., 1996; Dobson et al., 2008; Dobson et al., 2010; Silva et al., 2005). In this framework, we have decided to verify whether free amino acid profiles could be used to characterize Annurca apples from four territories of Campania Region. This approach represents the first application of free amino acid profiles to identify the geographic origin of the Annurca apple.

The analysis of Annurca apples led to the identification of different clusters corresponding to the four districts (Fig 5). The plot obtained with this PCO analysis (Fig 7) made it possible to group the Annurca apples on the basis of the geographical origin. The confidence of the cluster obtained for *M. domestica* apples, was tested considering the free amino acid profiles of other vegetables (*A. officinalis*, *A. acutifolius*, *C. scolymus* cv Capuanella and *S. tuberosum* from various Italian districts and commercial cv Merit and cv Agria). The cluster (Fig 6) and PCO analyses (Fig 7) confirm that the free amino acid profiles from the analyzed samples segregate into well-defined groups.

In this study we propose a simple method, which could be transferred to other laboratories, for the determination, in food samples, of all free amino acids. The data collected on other plant species could be used to obtain clusters related to their cultivation district. A development of this study could consist in the characterization of Italian Annurca apples and of apple samples from other countries and in the extension of the study to processed products like juice and syrup.

Acknowledgments

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References

Andersen PC, Hill K, Gorbet DW, Brodbeck BV (1998) Fatty Acid and Amino Acid Profiles of Selected Peanut Cultivars and Breeding Lines. J Food Comp Anal 11:100-111

Baudoin JP, Maquet A (1999) Improvement of protein and amino acid contents in seeds of food legumes. A case study in Phaseolus. Biotechnol Agron Soc Environ 3 (4):220-224

Bruckner H, Westhauser T (2003) Chromatographic determination of L- and D-amino acids in plants. Amino Acids 24 (1-2):43-55

Burstin J, Charcosset A (1997) Relationship between phenotypic and marker distances: theoretical and experimental investigations. Heredity 79:477-483

Davies AMC (1976) The application of amino acid analysis to the determination of the geographical origin of honey. Int J Food Sci Tech 11:515-523

Davis JC (1986) Statistics and Data Analysis in Geology. John Wiley & Sons, New York.

Devitt DA, Stolzy LH, Labanauskas CK (1987) Impact of potassium, sodium, and salinity on the protein-and free amino acid content of wheat grain Plant and Soil 103 (1):101-109

Dobson G, Shepherd T, Verrall SR, Conner S, McNicol JW, Ramsay G, Shepherd LV, Davies HV, Stewart D (2008) Phytochemical diversity in tubers of potato cultivars and landraces using a GC-MS metabolomics approach. J Agric Food Chem 56 (21):10280-10291

Dobson G, Shepherd T, Verrall SR, Griffiths WD, Ramsay G, McNicol JW, Davies HV, Stewart D (2010) A metabolomics study of cultivated potato (*Solanum tuberosum*) groups Andigena, Phureja, Stenotomum, and tuberosum using gas chromatography-mass spectrometry. J Agric Food Chem 58 (2):1214-1223

Elbert EM, Esselen WB (2006) Free amino acids and thiamine of apple juices and their relation to fermentation. J Food Sci 24 (4):352-361

Faisal Anwar Malik M, Qureshi AS, Muhammad Ashraf M, Rashid Khan M, Asif Javed A (2009) Evaluation of genetic diversity in soybean (*Glycine max*) lines using seed protein electrophoresis. Aust J Crop Sci 3(2):107-112

Floris R (1997) Melo e pero nella frutticoltura italiana ed europea. In: Federchimica Agrofarma. Milano, Italy, p 17

Foyer CH, Parry M, Noctor G (2003) Markers and signals associated with nitrogen assimilation in higher plants. J Exp Bot 54 (382):585-593

Fritz C, Mueller C, Matt P, Feil R, Stitt M (2006) Impact of the C-N status on the amino acid profile in tobacco source leaves. Plant Cell Environ 29 (11):2055-2076

Good AG, Zaplachinski ST (1994) The effect of drought stress on fre amino acid accumulation and protein syntesis in Brassica napus Physiol Plant 90:9-14

Helena Seron L, Garrigòs Poveda E, Prats Moya MS, Martìn Carratalà ML, Berenguer-Navarro V, Granè -Teruel N (1998) Characterisation of 19 almond cultivars on the basis of their free amino acids composition. Food Chem 61 (4):455-459

Iriti M, Di Maro A, Bernasconi S, Burlini N, Simonetti P, Picchi V, Panigada C, Gerosa G, Parente A, Faoro F (2009) Nutritional traits of bean (*Phaseolus vulgaris*) seeds from plants chronically exposed to ozone pollution. J Agric Food Chem 57 (1):201-208

Iriti M, Rossoni M, Borgo M, Ferrara L, Faoro F (2005) Induction of resistance to gray mold with benzothiadiazole modifies amino acid profile and increases proanthocyanidins in grape: primary versus secondary metabolism. J Agric Food Chem 53 (23):9133-9139

Jacobsen T, Gunderson RW (1986) Applied cluster analysis, vol 361-408. Statistical Procedures in Food Research. Elsevier Science, New York

Kumar P, Gupta VK, Misra AK, Modi DR, Pandey BK (2009) Potential of Molecular Markers in Plant Biotechnology. Plant Omics J 2(4):141-162

Kyoung Rae K, Jung Han K, Eun-Ji C, Chan-min J (1996) Gas chromatographic amino acid profiling for pattern recognition. . J Chromatogr A 722:303-309

Martinez I, Aursand M, Erikson U, Singstad TE, Veliyulin E, van der Zwaag C (2003) Destructive and non-destructive analytical techniques for authentication and composition analyses of foodstuffs. Trends Food Sci Technol 14:489-498.

Melchiade D, Foroni I, Corrado G, Santangelo I, Rao R (2007) Authentication of the 'Annurca' Apple in Agro-food Chain by Amplification of Microsatellite Loci. Food Biotechnol 21:33-43

Merken HM, Beecher GR (2000) Measurement of Food Flavonoids by High-Performance Liquid Chromatography: A Review. J Agric Food Chem 48:577-599

Pilando LS, Worlstad RE (1992) The effectiveness of pattern recognition, sugar, nonvolatile acid, and $^{13}C/^{12}C$ analyses for detecting adulteration in apple juice. J Food Comp Anal 5:10-24

Sano M, Kawashima N (1982) Changes in the Contents of Free and Protein Amino Acids in Tobacco Seeds and Placentae during Maturation. Plant Cell Physiol 23:1245 - 1250

Silva BM, Andrade PB, Martins RC, Valentao P, Ferreres F, Seabra RM, Ferreira MA (2005) Quince (*Cydonia oblonga* Miller) fruit characterization using principal component analysis. J Agric Food Chem 53 (1):111-122

Soufleros EH, Bouloumpasi E, Tsarchopoulos C, Biliaderis CG (2003) Primary amino acid profiles of Greek white wines and their use in classification according to variety, origin and vintage. Food Chem 80:261-273

Stein WH, Moore S (1963) Chomatographic determination of amino acids by the use of automatic recording equipment. Methods Enzymol 6:819-831

Theocharis A, Hand P, Pole J, Cevik V, Fisarakis I, Henderson J (2010) Study of genetic diversity among inter-intraspesific hybrids and original grapevine varieties using AFLP molecular markers. Aust J Crop Sci 4(1):1-8

Tomàs X, Molins JJ (1990) Aplicaciòn de tècnicas quimiomètricas al estudio de la fracciòn esteròlica del cafè. Afinidad 47:179-184

Vannini C, Iriti M, Bracale M, Locatelli F, Faoro F, Croce P, Pirona R, Di Maro A, Coraggio I, Genga A (2006) The ectopic expression of the rice Osmyb4 gene in Arabidopsis increases tolerance to abiotic, environmental and biotic stresses. Physiol Mol Plant Pathol 69:26-42

Varela P, Pablos F, Gonzàlez AG (1996) Classification of tea samples by their chemical composition using discriminant analysis. Talanta 43:415-419

Vasanits A, Kutlan D, Sass P, Molnar-Perl I (2000) Retention/quantitation properties of the o-phthaldialdehyde-3-mercaptopropionic acid and the o-phthaldialdehyde-N-acetyl-L-cysteine amino acid derivatives in reversed-phase high-performance liquid chromatography. J Chromatogr A 870 (1-2):271-287

Vasanits A, Molnar-Perl I (1999) Temperature, eluent flow-rate and column effects on the retention and quantitation properties of phenylthiocarbamyl derivatives of amino acids in reversed-phase high-performance liquid chromatography. J Chromatogr A 832 (1-2):109-122