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A multivariate statistical analysis coming from the NMR metabolic profile of cherry tomatoes (The Sicilian Pachino case)

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HIGHLIGHTS

- We use the HR-MAS NMR technique in order to study the metabolic profile of the Sicilian cherry tomato of Pachino.
- We determine the molar concentration of the metabolites present in the cherry tomato of Pachino.
- We make use of multivariate statistical analysis for the discrimination of those metabolites that are responsible for sample differentiation.
- Cherry tomatoes of Pachino have a higher concentration of sugars, essential aminoacids and GABA.
- Cherry tomatoes of Pachino have a lower concentration of methanol and fatty acids.

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ABSTRACT

We have studied by means of High Resolution Magic Angle Spinning Nuclear Magnetic Resonance the metabolic profile of the famous Sicilian cherry tomato of Pachino. Thanks to its organoleptic and healthy properties, this particular foodstuff was the first tomato accredited by the European PGI (Protected Geographical Indication) certification of quality. Due to the relatively high price of the final product commercial frauds originated in the Italian and international markets. Hence, there is a growing interest to develop analytical techniques able to predict the origin of a tomato sample, indicating whether or not it originates from the area of Pachino, Sicily (Italy). In this paper we have determined the molar concentration of the metabolites constituent the PGI cherry tomato of Pachino. Furthermore, by means of a multivariate statistical analysis we have identified which metabolites are relevant for sample differentiation.

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1. Introduction

In the area of food sciences, food characterization is a very challenging topic because it includes both authenticity and geographical origin determination [1]. In fact, in the literature there is a large amount of studies about the development of analytical methods aimed to characterize in an unambiguous way a particular foodstuff [2].

The most suitable approach in the field of biological systems seems to be Metabolomics, the scientific discipline able to identify and quantify the different metabolites that determine and characterize the studied bio-system in its particular state [3]. In fact, since metabolism represents the physical and chemical reactions that occur in a living organism (or part thereof), and consists essentially in transformations of matter caused by energetic variations, the metabolic content is important for a comprehensive analysis of all metabolite changes upon stimuli [3] or stress conditions [4]. Hence, the precise metabolite content constitutes a sort of fingerprint for a given bio-system in its particular state that in principle can be used for its unambiguous identification. Metabolomics takes advantage of multivariate statistical approaches in order to evaluate large sets of information obtained by advanced analytical techniques and to discriminate information dealing with redundancy often present in metabolomics data [5]. The most used techniques for metabolic profiling are mass-spectrometry (MS) coupled with liquid or gas chromatographic separation (LC/MS and principally GC/MS) and NMR spectroscopy [6–11]. Even if NMR spectroscopy is less sensitive than MS techniques, is a rapid, non-destructive, high-throughput method for identifying and quantifying plant metabolites [12–15].

Probably the most widely consumed fresh vegetable is tomato (Solanum lycopersicum) which is an important component of healthy diets and in particular of the Mediterranean one. The active properties of tomato depend on its relatively high concentration of, apart from sugars, lycopene, ascorbic acid, vitamin E, flavonoids, etc. Tomatoes are low in calories and possess antioxidant, antitumoral and antidepressive properties [16,17] so there is an increasing demand of quality control by consumers.

The Sicilian cherry tomato of Pachino was the first tomato accredited by the PGI certificate, a European certification that guarantees the geographical origin of a product and specific quality criteria [18,19]. Its peculiar organoleptic and healthy properties are linked to its taste, which is in turn related with the amount of sugars, organic acids, free amino acids and salts [20]. The production area of the cherry tomato of Pachino includes the entire municipality of Pachino and Portopalo and part of the territories of Noto and Ispica, located in the south east of Sicily (Italy). In this land the cultivation of horticultural products has found its ideal soil and climate conditions due to high exposure to the sun, the salinity of the irrigation water, the soil consistency and the proximity of the sea, resulting in climate mitigation and a low frequency of winter-spring frosts. Therefore, in order to protect both producers and consumers from the increasing number of frauds present in the Italian and international markets, an exhaustive metabolic profile of the Sicilian cherry tomato of Pachino is essential. Furthermore, the metabolites quantification could be an additional *weapon* against frauds.

In this manuscript we present a proton nuclear magnetic resonance (^{1}H NMR) study of the PGI cherry tomato of Pachino. By means of the experimental technique known as High Resolution Magic Angle Spinning (HR-MAS) we were able to identify and quantify the main metabolites constituting this protected tomato species.

The HR-MAS technique represents a recent and extremely powerful NMR method allowing for simultaneous analysis of both polar and apolar components within micro quantities of semi-solid food samples without requiring separation or extraction procedures [21–23]. In fact, by means of HR-MAS, biological samples such as cells, DNA, proteins, intact tissues, organs, and membranes can be efficiently studied in normal or altered conditions [4,24–27]. Furthermore, this technique was recently applied also to study the degradation pathways of the ancient paper [28].

In the last years different NMR studies [29–33] were addressed to metabolic profiling of tomato for tissue differentiation and fruit ripening [30,31] or for discrimination analyses [31,32]. Interesting results concern the abundance of tryacylglycerols in seeds and of fructose, glucose, citric acid, 4-aminobutyric acid (GABA), glutamine and glutamate in flesh, peel and tomato purée [30]. For what concern sample differentiation, Consonni and coworkers were able to discriminate between triple concentrated tomato paste coming from Italy and China [32]. Citrate content resulted in being the most relevant chemical compound for Chinese and Italian sample differentiation together with aspartate, glutamine, and sugars concentration. Furthermore sample discrimination was performed to detect potential unintended effects following genetic modifications [31].

The aim of this work is the use of the HR-MAS technique in order to determine the molar concentration of the major content of metabolites present in the cherry tomato of Pachino. Furthermore we make use of multivariate statistical analysis for the discrimination of those metabolites responsible for sample differentiation.

2. Materials and methods

In order to avoid metabolic differences due to different ripening stage [30,31,34], all tomato samples were studied in the red stage, that is when more than 90% of the surface, in the aggregate, is red. In particular, we considered 14 different kinds of cherry tomato samples of Pachino, 12 cherry tomato samples of dubious provenience (not Pachino) and only 2 coming directly from Beijing (China). For a statistically significant outcome we analyzed at least 5 samples for each kind of tomato. The PGI cherry tomatoes of Pachino were provided by *Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri"* (http://www.izssicilia.it/). The sample preparative is relatively simple: 6 mg of freeze-dried tomato were diluted in 100 μl of D_2O containing 1 mM 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) and then vortexed for a couple of minutes. Finally,