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Monitoring of metabolic profiling and water status of Hayward kiwifruits by nuclear magnetic resonance☆

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ABSTRACT

The metabolic profiling of kiwifruit (*Actinidia deliciosa*, *Hayward* cultivar) aqueous extracts and the water status of entire kiwifruits were monitored over the season (June–December) using nuclear magnetic resonance (NMR) methodologies. The metabolic profiling of aqueous kiwifruit extracts was investigated by means of high field NMR spectroscopy. A large number of water-soluble metabolites were assigned by means of 1D and 2D NMR experiments. The change in the metabolic profiles monitored over the season allowed the kiwifruit development to be investigated. Specific temporal trends of aminoacids, sugars, organic acids and other metabolites were observed.

The water status of kiwifruits was monitored directly on the intact fruit measuring the T_2 spin–spin relaxation time by means of a portable unilateral NMR instrument, fully non-invasive. Again, clear trends of the relaxation time were observed during the monitoring period.

The results show that the monitoring of the metabolic profiling and the monitoring of the water status are two complementary means suitable to have a complete view of the investigated fruit.

1. Introduction

The increasing ability of high field NMR spectroscopy to solve spectra of complex mixtures and to recognize and quantify each component without chemical separation, has found a constantly increasing application in metabolomics and food chemistry [1]. ^{1}H high field NMR spectroscopy has shown to be a valuable tool for the qualitative and quantitative analysis of the metabolic profiling of food stuff such as truffles [2], sea bass [3] olive oils [4], tomatoes [5], lettuce [6] and mangoes [7]. The quantitative analysis of the metabolic profiling along with the application of a suitable statistical analysis has allowed food characterization in terms of geographical origin [8], genetic origin [9–14] and farming [3]. The potential of NMR spectroscopy to detect food adulterations has been also demonstrated [15].

In recent years kiwifruit has become an important horticultural crop. For the consumer, desirable attributes of kiwifruit are flavour, fragrance and healthful properties which are obviously due to the fruit chemical composition. In particular, the flavour of the fruit flesh is highly dependent on the balance between soluble sugars and non-volatile organic acids. Besides, different sugars are responsible of different sweetness levels whereas organic acids give a different perception of the acidity. Consequently, qualitative and quantitative analyses of metabolites in kiwifruits are very

Abbreviations: AA, ascorbic acid; ATP, adenosine tri-phosphate; ALA, alanine; ARG, arginine; ASN, asparagine; ASP, aspartate; CHN, choline; CA, citric acid; COSY, correlated spectroscopy; CPMG, Carr Purcell Meiboom Gill; DOSY, diffusion ordered spectroscopy; αFRUfu, α-D-fructofuranose; βFRUfu, β-D-fructofuranose; βFRUpy, β-D-fructopyranose; FRU6P, fructose-6-phosphate; αGAL, α-galactose; βGAL, β-galactose; GAL-U, galactose-U; GARP, globally optimized alternating phase rectangular pulse; GLUtCA, O^3-β-D-glucopyranosyl-*trans*-caffeic acid; GLUcCA, O^3-β-D-glucopyranosyl-*cis*-caffeic acid; αGLC, α-glucose; βGLC, β-glucose; αGLC6P, α-glucose-6-phosphate; βGLC6P, β-glucose-6-phosphate; GLU, glutamate; GLN, glutamine; HMBC, heteronuclear multiple-bond correlation; HSQC, heteronuclear single quantum coherence; ILE, isoleucine; LA, lactic acid; LEU, leucine; LYS, lysine; MA, malic acid; αMAN, α-mannose; βMAN, β-mannose; MI, *myo*-inositol; NOESY, nuclear overhauser and exchange spectroscopy; PGSE, pulsed field gradient spin echo; QA, quinic acid; RAF, raffinose; RIB, ribose; SHA, shikimic acid; SUCR, sucrose; THR, threonine; TOCSY, total correlation spectroscopy; TRP, triptophane; TSP, trimethylsilylpropionate; URI, uridine; VAL, valine; αXYL, α-xylose; βXYL, β-xylose.

☆ In memory of Annalaura Segre, our beloved teacher and friend.

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important for the commercial success. The knowledge of nutritional profile of kiwifruits is also extremely important for the industries which extract specific compounds from the fruit to obtain additives for other foodstuffs.

In this framework the knowledge of the metabolic profiling of kiwifruits at different development stages may have an important role in the determination of the most suitable time of harvesting as well as in the quantitative determination of nutrients at different growth times. Various results have been previously reported on the kiwifruit chemical composition using different techniques [16,17]. In particular, HPLC technique has allowed the determination, in kiwi juice, of some classes of compounds such as organic acids, sugars and amino acids [18–20]. ^{1}H NMR spectroscopy has been previously applied for the quantitative analysis of malic and citric acids in kiwifruit juice samples at adjusted pH values [21]. However, at our knowledge, a full high field NMR study for the determination of the metabolic profiling of kiwifruit has not been reported in the literature.

In the present paper, we report an NMR investigation of kiwifruit (*Hayward* cultivar) aqueous extracts. As a non-specific high-throughput analytical method, NMR spectroscopy is well suited to the requirements of metabolic profiling having the advantage to detect signals due to many different classes of compounds in the same experiment. The metabolic profiling of aqueous extracts monitored over a seven months period was determined to investigate the kiwifruit composition at different harvesting times.

Along with a detailed high field NMR study, a low field non-invasive ^{1}H NMR investigation of kiwifruits is also reported. As well known, low field ^{1}H NMR relaxometry is an important tool to investigate the water status in foodstuffs [22]. In fact this methodology allows to obtain information on water compartments, diffusion, and movement, detecting protons predominantly contributed by ^1H_2O contained in foodstuffs. In literature, different foodstuffs, such as mozzarella cheese [23], banana fruits [24], and meat [25] have been investigated by means of low field NMR and important information on the food texture and on the ripening status has been obtained.

It has been shown that the novel recently available low field portable NMR instruments [26,27] provide information on the entire object *in situ* and even if the magnetic field penetrating the object is rather inhomogeneous, useful information can be obtained in many fields of application such as soft matter, plant leaves [28], biological tissues and porous materials [29,30]. Here, we report the water status of the entire kiwifruits monitored over the season by means of a portable unilateral NMR instrument.

2. Materials and methods

2.1. Materials

Hayward kiwifruits were hand harvested in an experimental field located in Lazio region, Italy, over a period beginning in June 2008 and ending in December 2008 carrying out seven campaigns of measurement to monitor a wide range of developing. It is important to point out that the same kiwifruits were used to perform both low field NMR measurements with portable instrument immediately after the harvesting and high field NMR measurements on the aqueous extracts.

2.2. High-resolution NMR measurements

2.2.1. Samples preparation

Fresh cut pulp (1 g) was frozen in liquid N_2, finely powdered, and submitted to an extraction according to the modified [31] Bligh–Dyer methodology [32] with methanol/chloroform/water in 2:2:1 volumetric ratio. Sample was kept at 4 °C for 1 h and then centrifuged for 20 min at 11,000 × *g* (times gravity) at 4 °C. The upper hydroalcolic phase and the lower organic phase were carefully separated and dried under an N_2 flow. The dried phases were stored at −80 °C until the NMR analysis.

2.2.2. NMR spectra

The dry residue of the hydroalcolic phase was dissolved in a D_2O phosphate buffer (100 mM, pH 7.2) containing 3-(trimethylsilyl)-propionic-2,2,3,3,-d_4 acid sodium salt (TSP, 2 mM) as internal standard.

The NMR spectra of kiwifruit aqueous extracts were recorded at 27 °C on a Bruker AVANCE AQS600 spectrometer operating at the proton frequency of 600.13 MHz and equipped with a Bruker multinuclear *z*-gradient inverse probehead. ^{1}H spectra were referenced to TSP signal (δ = 0.00 ppm) whereas ^{13}C spectra were referenced to the CH-1 resonance of α-D-glucose (δ = 93.10 ppm).

The ^{1}H spectra of the aqueous extracts were acquired by co-adding 512 transients with a recycle delay of 3 s and using a 90° pulse of 10.8 μs, 32K data points. The water signal was suppressed using a solvent presaturation (NOESY-presaturation scheme) during the relaxation delay and a mixing time of 160 ms [33]. In order to minimize the variability of the signals intensity due to water suppression, a careful calibration of the soft pulse for water suppression was always performed.

2D NMR experiments, namely ^{1}H–^{1}H COSY, ^{1}H–^{1}H TOCSY, ^{1}H–^{1}H NOESY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC [33], were performed using the same experimental conditions previously reported [2]. The mixing time for the ^{1}H–^{1}H TOCSY was 80 ms, the mixing time for ^{1}H–^{1}H NOESY was 400 ms. The HSQC experiments were performed using a coupling constant $^1J_{C-H}$ of 150 Hz and the ^{1}H–^{13}C HMBC experiments were performed using a delay for the evolution of long-range couplings of 80 ms.

The {^{1}H}-decoupled ^{31}P NMR experiments were performed at 242.94 MHz by co-adding 1500 transients with a recycle delay of 7 s, a 20 kHz spectral width, 8K data points, a GARP pulse sequence for proton decoupling, and a 90° ^{31}P pulse of 14 μs. Chemical shifts for the ^{31}P spectrum were given in ppm with respect to an external standard of 85% H_3PO_4.

The ^{1}H–^{31}P HMBC spectra were obtained using a recycle delay of 2 s, a 90° ^{1}H pulse of 11 μs and a 90° ^{31}P pulse of 14 μs and 6 and 10 kHz spectral widths in proton (F2) and phosphorus (F1) dimensions respectively, 1K data points in F2, 512 increments in F1, and a linear prediction up to 1K points in F1. Data were processed using un-shifted sinusoidal window functions in both dimensions. The delay for the evolution of long-range couplings was 80 ms.

Pulsed field gradient spin echo (PGSE) experiments [34,35] were performed with a pulsed field gradient unit producing a magnetic field gradient in the *z*-direction with a strength of 55.4 G cm^{-1}. The stimulated echo pulse sequence using bipolar gradients with a longitudinal eddy current delay was used. The strength of the sine-shaped gradient pulse with a duration of 1.4 ms, was logarithmically incremented in 32 steps, from 2% up to 95% of the maximum gradient strength, with a diffusion time of 120 ms and a longitudinal eddy current delay of 25 ms. After Fourier transformation and a baseline correction, the diffusion dimension was processed using the DOSY [36,37] subroutine of the Bruker TOPSPIN 1.3 software package.

The detection limit of a given metabolite, analyzed in a 5 mm tube using 1D ^{1}H NMR spectroscopy at high field (11–16 T) is about 50 μM.

2.2.3. Statistical analysis

In order to perform a comparison between ^{1}H spectra of different kiwifruits, the signal linewidth of each specific resonance has to be the same in all analyzed spectra. It can be obtained by using